(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) **EP 1 666 028 B1**

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
24.03.2010 Bulletin 2010/12

(51) Int Cl.:
A61K 9/12 *(2006.01)* ***A61K 47/18*** *(2006.01)*

(21) Application number: **05027610.4**

(22) Date of filing: **12.04.2000**

(54) **Dry powder compositions having improved dispersivity**

Trockenpulverzusammensetzungen mit verbesserter Dispersität

Compositions de poudre sèche à dispersivité ameliorée

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

(30) Priority: **29.10.1999 US 162451 P**
08.11.1999 US 164236 P
20.12.1999 US 172769 P
27.01.2000 US 178383 P
27.01.2000 US 178415 P

(43) Date of publication of application:
07.06.2006 Bulletin 2006/23

(62) Document number(s) of the earlier application(s) in accordance with Art. 76 EPC:
00922117.7 / 1 223 915

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(56) References cited:
WO-A-96/21461 **WO-A1-97/03649**

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EP 1 666 028 B1

Printed by Jouve, 75001 PARIS (FR)

Description

[0001] This application claims the benefit of priority of the following U.S. Provisional Patent Applications: Serial No. 60/162,451 filed on October 29, 1999; Serial No. 60/164,236 filed on November 8, 1999, Serial No. 60/172,769 filed on December 20, 1999; Serial No. 60/178,383 filed on January 27, 2000; and Serial No. 60/178,415 filed on January 27, 2000.

[0002] The present invention is directed to highly dispersive dry powder compositions, and in particular, to highly dispersive, inhalable dry powder compositions for aerosolized delivery to the lungs. The dry powders of the invention contain an active agent and a di- or tripeptide containing at least 2 leucyl residues, and are physically and chemically stable upon storage. The powders of the invention also demonstrate superior aerosol performance.

[0003] Traditionally, inhalation therapy has played a relatively minor role in the administration of biotherapeutics and conventional pharmaceuticals when compared to more traditional drug administration routes, such as oral and intraveneous. Injection is the customary route of delivery of biotherapeutics (e.g., peptides, proteins and nucleic acids), and due to the many drawbacks associated with injection (e.g., inconvenience, discomfort, patient aversion to needle-based delivery methods), alternative administration routes are needed.

[0004] Pulmonary delivery is one such alternative administration route which can offer several advantages over subcutaneous administration. These advantages include the convenience of patient self-administration, the potential for reduced drug side-effects, ease of delivery by inhalation, the elimination of needles, and the like. Many preclinical and clinical studies with inhaled proteins, peptides, DNA and small molecules have demonstrated that efficacy can be achieved both within the lungs and systemically. However, despite such results, the role of inhalation therapy in the health care field has not grown as expected over recent years, in part due to a set of problems unique to the development of inhalable drug formulations. Dry powder formulations, while offering unique advantages over cumbersome liquid dosage forms and propellant-driven formulations, are prone to aggregation and low flowability phenomena which considerably diminish the efficiency of dry powder-based inhalation therapies.

[0005] Particulate aggregation, caused by particle-particle interactions, such as hydrophobic, electrostatic, and capillary interactions, must be minimized in order to provide dispersible powders for effective inhalation therapies. Various approaches have been utilized in efforts to prepare dry powders having minimal particle aggregation and good aerosol properties. These approaches include the modification of dry powder particle surface texture (Ganderton, et al., U.S. Pat. No. 5,376,386), the co-delivery of large carrier particles (absent drug) with therapeutic aerosols to achieve efficient aerosolization, particle coatings (Hanes, 5,855,913; Ruel, et al., 5,663,198), aerodynamically light particles (Edwards, et al., 5,985,309), use of antistatic agents, (Simpkin, et al., 5,908,639) and the addition of certain excipients, e.g., surfactants (Hanes 5,855,913; Edwards, 5,985,309). Unfortunately, the formation of particulate aggregates and production of powders having poor flow properties and low dispersivities continue to plague development efforts to prepare aerosolizable dry powders for inhalation therapy. Thus, a need exists for improved inhalable aerosols for the pulmonary delivery of therapeutic agents, and in particular, for dry powders having excellent aerosol properties and reduced particle-particle interactions, irrespective of the therapeutic agent.

[0006] The introduction of an anti-adherent material, in particular leucine, into powders for use in dry powder inhalers, has been disclosed in WO 97/03649.

[0007] A tripeptide containing two leucine moieties, namely Leu-His-Leu has been used according to WO 96/21461 to provide stable pharmaceutical formulations comprising a growth hormone.

[0008] The present invention is based upon the discovery of a particular class of excipients, which, when incorporated into dry powder formulations for aerosolization and delivery to the lung, notably improves the dispersivity and aerosolization properties of the dry powders, irrespective of the type of active agent contained in the formulation. More particularly, the invention provides a dry powder composition as defined in the annexed claims which comprises an active agent and a di or tri-peptide comprising at least two leucines. Preferred di- and tripeptides are those which are surface active.

[0009] The dry powder of the invention typically contains from 2% by weight to 99% by weight di- or tri-peptide, and may optionally contain additional excipients or carriers, such as carbohydrates, amino acids, peptides, proteins, organic acid salts, and/or polymers.

[0010] The presence of the di- or tri-peptide is effective to notably increase the emitted dose of the dry powder over the emitted dose of the powder composition absent the di- or tri-peptide. In one particular embodiment of the invention, the dry powder of the invention is characterized by an emitted dose of at least 30%. In another embodiment, the concentration of the dileucyl- di- or tri-peptide on the surface of the particles is greater than in the bulk powder.

[0011] Additional features of the dry powder particles of the invention include, in one embodiment, a mass median diameter of less than about 10 microns, and in yet another embodiment, a mass median aerodynamic diameter of less than about 10 microns. In yet another embodiment, the dry powder comprises particles having a bulk density from 0.1 to 10 grams per cubic centimeter.

[0012] The dry powder of the invention is further characterized by both physical and chemical stability upon storage, as characterized, in one embodiment, by a drop in emitted dose of no more than 10% when stored under ambient conditions for a period of three months. In another embodiment, the chemical stability of the dry powder is characterized

by degradation of less than 5% by weight of the active agent upon storage of the dry powdered composition under ambient conditions for a period of three months.

[0013] In yet a further aspect, the invention provides a method for increasing the aerosol performance of an active-agent containing formulation as defined in the annexed claims suitable for administration to the lung. According to the method, a di- or tripeptide comprising at least two leucines is incorporated into a formulation comprising an active agent. The resulting composition comprising the active agent and the di- or tripeptide possesses an emitted dose that is increased over the emitted dose of a composition having the same components but absent the di- or tripeptide. In one embodiment, the method results in a liquid composition suitable for aerosolized administration to the lung; in an alternative embodiment, the method results in a dry powdered composition suitable for aerosolized administration to the lung.

[0014] Yet another aspect of the invention is directed to the use of a liquid formulation produced by the above defined method for the preparation of a medicament for delivery of a dry powder composition to the lungs of a mammalian subject by administering by inhalation the compositions of the invention as previously described, in aerosolized form.

[0015] These and other objects and features of the invention will become more fully apparent when the following detailed description is read in conjunction with the accompanying figures and examples.

Detailed Description of the Invention

I. Definitions

[0016] The following terms as used herein have the meanings indicated.

[0017] "Active agent" as described herein includes any agent, drug, compound, composition of matter or mixture which provides some pharmacologic, often beneficial, effect that can be demonstrated *iri-vivo* or *in vitro*. This includes foods, food supplements, nutrients, nutriceuticals, drugs, vaccines, antibodies, vitamins, and other beneficial agents. As used herein, these terms further include any physiologically or pharmacologically active substance that produces a localized or systemic effect in a patent.

[0018] "Amino acid" refers to any compound containing both an amino group and a carboxylic acid group. Although the amino group most commonly occurs at the position adjacent to the carboxy function, the amino group may be positioned at any location within the molecule. The amino acid may also contain additional functional groups, such as amino, thio, carboxyl, carboxamide, imidazole, etc. An amino acid may be synthetic or naturally occurring, and may be used in either its racemic or optically active (D-, or L-) form

[0019] "Leucine", whether present as a single amino acid or as an amino acid component of a peptide, refers to the amino acid leucine, which may be a racemic mixture or in either its D- or L- form, as well as modified forms of leucine (i.e., where one or more atoms of leucine have been substituted with another atom or functional group) in which the dispersibility-enhancing effect of the modified amino acid or peptide is substantially unchanged or unimproved over that of the unmodified material.

[0020] "Dipeptide", also referred to herein as a dimer, refers to a peptide composed of two amino acids.

[0021] "Tripeptide", also referred to herein as a trimer, refers to a peptide composed of three amino acids.

[0022] A "surface active" material is one having surface activity (measured, e.g., by surface tensiometry), as characterized by its ability to reduce the surface tension of the liquid in which it is dissolved. Surface tension, which is associated with the interface between a liquid and another phase, is that property of a liquid by virtue of which the surface molecules exhibit an inward attraction.

[0023] Typically, in the context of the present invention, a surface active dipeptide or tripeptide is identified by preparing solutions of varying concentrations (from approximately 0.01% wt/vol (0.1 mg/ml) to approximately 2% wt/vol (20 mg/ml) of the subject peptide in water, and measuring the surface tension of each of the solutions. A surface-active peptide is one which, when present at any concentration in solution, though typically present in an amount greater than 0.25 mg/ml, is effective to lower the surface tension of water from its control value. A peptide that is more surface active than another peptide is one which decreases the surface tension of water to a greater extent, when present in the liquid at the same concentration and measured under the same set of experimental conditions.

[0024] "Dry powder" refers to a powder composition that typically contains less than about 20% moisture, preferably less than 10% moisture, more preferably contains less than about 5-6% moisture, and most preferably contains less than about 3% moisture, depending upon the particular formulation.

[0025] A dry powder that is "suitable for pulmonary delivery" refers to a composition comprising solid (i.e., non-liquid) or partially solid particles that are capable of being (i) readily dispersed in/by an inhalation device and (ii) inhaled by a subject so that a portion of the particles reach the lungs to permit penetration into the alveoli. Such a powder is considered to be "respirable"

[0026] "Aerosolized" or "aerosolizable" particles are particles which, when dispensed into a gas stream by either a passive or an active inhalation device, remain suspended in the gas for an amount of time sufficient for at least a portion of the particles to be inhaled by the patient, so that a portion of the particles reaches the lungs.

[0027] "Emitted Dose" or "ED" provides an indication of the delivery of a drug formulation from a suitable inhaler device after a firing or dispersion event. More specifically, for dry powder formulations, the ED is a measure of the percentage of powder which is drawn out of a unit dose package and which exits the mouthpiece of an inhaler device. The ED is defined as the ratio of the dose delivered by an inhaler device to the nominal dose (*i.e.*, the mass of powder per unit dose placed into a suitable inhaler device prior to firing). The ED is an experimentally-determined parameter, and is typically determined using an *in-vitro* device set up which mimics patient dosing. To determine an ED value, a nominal dose of dry powder, typically in unit dose form, is placed into a suitable dry powder inhaler (such as that described in U.S. Patent No. 5,785,049, assigned to Inhale Therapeutic Systems) which is then actuated, dispersing the powder. The resulting aerosol cloud is then drawn by vacuum from the device, where it is captured on a tared filter attached to the device mouthpiece. The amount of powder that reaches the filter constitutes the emitted dose. For example, for a 5 mg dry powder-containing dosage form placed into an inhalation device, if dispersion of the powder results in the recovery of 4 mg of powder on a tared filter as described above, then the emitted dose for the dry powder composition is: 4 mg (delivered dose)/5 mg (nominal dose) x 100 = 80%. For non-homogenous powders, ED values provide an indication of the delivery of drug from an inhaler device after firing rather than of dry powder, and are based un amount of drug rather than on total powder weight. Similarly for MDI and nebulizer dosage forms, the ED corresponds to the percentage of drug which is drawn from a dosage form and which exits the mouthpiece of an inhaler device.

[0028] "Fine particle dose" or "FPD" is defined as the mass percent of powder particles having an aerodynamic diameter less than 3.3 μm typically determined by measurement in an Andersen cascade impactor. This parameter provides an indication of the percent of particles having the greatest potential to reach the deep lung of a patient for systemic uptake of a drug substance.

[0029] A "dispersible" or "dispersive" powder is one having an ED value of at least about 30%, more preferably 40-50%, and even more preferably at least about 50-60%.

[0030] "Mass median diameter" or "MMD" is a measure of mean particle size, since the powders of the invention are generally polydisperse (i.e., consist of a range of particle sizes). MMD values as reported herein are determined by centrifugal sedimentation, although any number of commonly employed techniques can be used for measuring mean particle size (e.g., electron microscopy, light scattering, laser diffraction).

[0031] "Mass median aerodynamic diameter" or "MMAD" is a measure of the aerodynamic size of a dispersed particle. The aerodynamic diameter is used to describe an aerosolized powder in terms of its settling behavior, and is the diameter of a unit density sphere having the same settling velocity, in air, as the particle. The aerodynamic diameter encompasses particle shape, density and physical size of a particle. As used herein, MMAD refers to the midpoint or median of the aerodynamic particle size distribution of an aerosolized powder determined by cascade impaction, unless otherwise indicated.

[0032] "Pharmaceutically acceptable salt" includes, but is not limited to, salts prepared with inorganic acids, such as chloride, sulfate, phosphate, diphosphate, hydrobromide, and nitrate salts, or salts prepared with an organic acid, such as malate, maleate, fumarate, tartrate, succinate, ethylsuccinate, citrate, acetate, lactate, methanesulfonate, benzoate, ascorbate, para-toluenesulfonate, palmoate, salicylate and stearate, as well as estolate, gluceptate and lactobionate salts. Similarly, salts containing pharmaceutically acceptable cations include, but are not limited to, sodium, potassium, calcium, aluminum, lithium, and ammonium (including alkyl substituted ammonium).

[0033] "Pharmaceutically acceptable excipient or carrier" refers to an excipient that may optionally be included in the compositions of the invention, and taken into the lungs with no significant adverse toxicological effects to the subject, and particularly to the lungs of the subject.

[0034] "Pharmacologically effective amount" or "physiologically effective amount of a bioactive agent" is the amount of an active agent present in an aerosolizable composition as described herein that is needed to provide a desired level of active agent in the bloodstream or at the site of action (e.g., the lungs) of a subject to be treated to give an anticipated physiological response when such composition is administered pulmonarily. The precise amount will depend upon numerous factors, e.g., the active agent, the activity of the composition, the delivery device employed, the physical characteristics of the composition, intended patient use (i.e., the number of doses administered per day), patient considerations, and the like, and can readily be determined by one skilled in the art, based upon the information provided herein.

[0035] "Polymer" refers to a high molecular weight polymeric compound or macromolecule built by the repetition of small, simple chemical units. A polymer may be a biological polymer, i.e., is naturally occurring (e.g., proteins, carbohydrates, nucleic acids) or a non-biological, synthetically-produced polymer (e.g., polyethylene glycols, polyvinylpyrrolidones, Ficolls, and the like), as well known in the art.

II. The Composition

[0036] The present invention is based upon the Applicants discovery of a class of compounds, dipeptides and tripeptides containing two or more leucines, which when incorporated into formulations for administration to the lung, impart superior

aerosol properties to the resulting formulations. Moreover, the Applicants have discovered, surprisingly that, these di- and tripeptides are effective to significantly enhance the dispersibility of the resulting formulations, irrespective of the type of active agent present in the formulation. Thus, these di- and tripeptides can be employed in a wide variety of formulations, to increase the aerosol performance of the resulting compositions, and in some cases, to provide aerosolizable formulations in situations where an aerosolizable formulation was previously unknown or unattainable. The present invention, although directed in certain respects to dry powder formulations, is meant to encompass liquid formulations as well. The components of the formulations of the invention will now be described.

A. The Active Agent

[0037] An active agent for incorporation in the compositions described herein may be an inorganic or an organic compound, including, without limitation, drugs which act on: the peripheral nerves, adrenergic receptors, cholinergic receptors, the skeletal muscles, the cardiovascular system, smooth muscles, the blood circulatory system, synaptic sites, neuroeffector junctional sites, endocrine and hormone systems, the immunological system, the reproductive system, the skeletal system, autacoid systems, the alimentary and excretory systems, the histamine system, and the central nervous system. Suitable agents may be selected from, for example, hypnotics and sedatives, psychic energizers, tranquilizers, respiratory drugs, anticonvulsants, muscle relaxants, antiparkinson agents (dopamine antagnonists), analgesics, antiinflammatories, antianxiety drugs (anxiolytics), appetite suppressants, antimigraine agents, muscle contractants, anti-infectives (antibiotics, antivirals, antifungals, vaccines) antiarthritics, antimalarials, antiemetics, antiepileptics, bronchodilators, cytokines, growth factors, anti-cancer agents, antithrombotic agents, antihypertensives, cardiovascular drugs, antiarrhythmics, antioxicants, anti-asthma agents, hormonal agents including contraceptives, sympathomimetics, diuretics, lipid regulating agents, antiandrogenic agents, antiparasitics, anticoagulants, neoplastics, antineoplastics, hypoglycemics, nutritional agents and supplements, growth supplements, antienteritis agents, vaccines, antibodies, diagnostic agents, and contrasting agents. The active agent, when administered by inhalation, may act locally or systemically.

[0038] The active agent may fall into one of a number of structural classes, including but not limited to small molecules, peptides, polypeptides, proteins, polysaccharides, steroids, proteins capable of eliciting physiological effects, nucleotides, oligonucleotides, polynucleotides, fats, electrolytes, and the like.

[0039] Examples of active agents suitable for use in this invention include but are not limited to calcitonin, erythropoietin (EPO), Factor VIII, Factor IX, ceredase, cerezyme, cyclosporin, granulocyte colony stimulating factor (GCSF), thrombopoietin (TPO), alpha-1 proteinase inhibitor, elcatonin, granulocyte macrophage colony stimulating factor (GMCSF), growth hormone, human growth hormone (HGH), growth hormone releasing hormone (GHRH), heparin, low molecular weight heparin (LMWH), interferon alpha, interferon beta, interferon gamma, interleukin-1 receptor, interleukin-2, interleukin-1 receptor antagonist, interleukin-3, interleukin-4, interleukin-6, luteinizing hormone releasing hormone (LHRH), factor IX insulin, pro-insulin, insulin analogues (e.g., mono-acylated insulin as described in U.S. Patent No. 5,922,675), amylin, C-peptide, somatostatin, somatostatin analogs including octreotide, vasopressin, follicle stimulating hormone (FSH), insulin-like growth factor (IGF), insulintropin, macrophage colony stimulating factor (M-CSF), nerve growth factor (NGF), tissue growth factors, keratinocyte growth factor (KGF), glial growth factor (GGF), tumor necrosis factor (TNF), endothelial growth factors, parathyroid hormone (PTH), glucagon-like peptide thymosin alpha 1, IIb/IIIa inhibitor, alpha-1 antitrypsin, phosphodiesterase (PDE) compounds, VLA-4 inhibitors, bisphosponates, respiratory syncytial virus antibody, cystic fibrosis transmembrane regulator (CFTR) gene, deoxyribonuclease (Dnase), bactericidal/permeability increasing protein (BPI), anti-CMV antibody, 13-cis retinoic acid, macrolides such as erythromycin, oleandomycin, troleandomycin, roxithromycin, clarithromycin, davercin, azithromycin, flurithromycin, dirithromycin, josamycin, spiromycin, midecamycin, leucomycin, miocamycin, rokitamycin, andazithromycin, and swinolide A, fluoroquinolones such as ciprofloxacin, ofloxacin, levofloxacin, trovafloxacin, alatrofloxacin, moxifloxacin, norfloxacin, enoxacin, grepafloxacin, gatifloxacin, lomefloxacin, sparfloxacin, temafloxacin, pefloxacin, amifloxacin, fleroxacin, tosufloxacin, prulifloxacin, irloxacin, pazufloxacin, clinafloxacin, and sitafloxacin, aminoglycosides such as gentamicin, netilmicin, paramecin, tobramycin, amikacin, kanamycin, neomycin, and streptomycin, vancomycin, teicoplanin, rampolanin, mideplanin, colistin, daptomycin, gramicidin, colistimethate, polymixins such as polymixin B, capreomycin, bacitracin, penems, penicillins including penicllinase-sensitive agents like penicillin G, penicillin V, penicllinase-resistant agents like methicillin, oxacillin, cloxacillin, dicloxacillin, floxacillin, nafcillin; gram negative microorganism active agents like ampicillin, amoxicillin, and hetacillin, cillin, and galampicillin; antipseudomonal penicillins like carbenicillin, ticarcillin, azlocillin, mezlocillin, and piperacillin; cephalosporins like cefpodoxime, cefprozil, ceftbuten, ceftizoxime, ceftriaxone, cephalothin, cephapirin, cephalexin, cephradrine, cefoxitin, cefamandole, cefazolin, cephaloridine, cefaclor, cefadroxil, cephaloglycin, cefuroxime, ceforanide, cefotaxime, cefatrizine, cephacetrile, cefepime, cefixime, cefonicid, cefoperazone, cefotetan, cefmetazole, ceftazidime, loracarbef, and moxalactam, monobactams like aztreonam; and carbapenems such as imipenem, meropenem, pentamidine isethiouate, albuterol sulfate, lidocaine, metaproterenol sulfate, beclomethasone dipropionate, triamcinolone acetamide, budesonide acetonide, fluticasone, ipratropium bromide, flunisolide, cromolyn sodium, ergot-

amine tartrate and where applicable, analogues, agonists, antagonists, inhibitors, and pharmaceutically acceptable salt forms of the above. In reference to peptides and proteins, the invention is intended to encompass synthetic, native, glycosylated, unglycosylated, pegylated forms, and biologically active fragments and analogs thereof.

[0040] Active agents for use in the invention further include nucleic acids, as bare nucleic acid molecules, vectors, associated viral particles, plasmid DNA or RNA or other nucleic acid constructions of a type suitable for transfection or transformation of cells, i.e., suitable for gene therapy including antisense. Further, an active agent may comprise live attenuated or killed viruses suitable for use as vaccines. Other useful drugs include those listed within the Physician's Desk Reference (most recent edition).

[0041] The amount of active agent in the formulation will be that amount necessary to deliver a therapeutically effective amount of the active agent per unit dose to achieve the desired result. In practice, this will vary widely depending upon the particular agent, its activity, the severity of the condition to be treated, the patient population, dosing requirements, and the desired therapeutic effect. The composition will generally contain anywhere from 1% by weight to 99% by weight active agent, typically from 2% to 95% by weight active agent, and more typically from 5% to 85% by weight active agent, and will also depend upon the relative amounts of additives contained in the composition. The compositions of the invention are particularly useful for active agents that are delivered in doses of from 0.001 mg/day to 100 mg/day, preferably in doses from 0.01 mg/day to 75 mg/day, and more preferably in doses from 0.10 mg/day to 50 mg/day.

[0042] It is to be understood that more than one active agent may be incorporated into the formulations described herein and that the use of the term "agent" in no way excludes the use of two or more such agents.

B. Dispersibility-Enhancing Peptides

[0043] Compositions of the invention will include one or more di- or tripeptides containing two or more leucine residues. As discussed above, the invention is based upon the Applicants discovery that dileucine as dipeptide and tripeptides as defined in the annexed claims are superior in their ability to increase the dispersibility of powdered compositions, and, as demonstrated in the Examples, are unexpectedly better than leucine in improving aerosol performance.

[0044] Di-leucyl containing tripeptides for use in the invention are tripeptides having the formula, X-Y-Z, where at least X and Y or X and Z are leucyl residues (i.e., the leucyl residues can be adjacent to each other (at the 1 and 2 positions), or can form the ends of the trimer (occupying positions 1 and 3). The remaining amino acid contained in the trimer can be any amino acid as defined in section I above. Suitable are amino acids such as glycine (gly), alanine (ala), valine (val), leucine (leu), isoleucine (ile), methionine (met), proline (pro), phenylalanine (phe), tryptophan (trp), serine (ser), threonine (thr), cysteine (cys), tyrosine (tyr), asparagine (asp), glutamic acid (glu), lysine (lys), arginine (arg), histidine (his), norleucine (nor), and modified forms thereof. Preferably, for di-leucyl containing trimers, the third amino acid component of the trimer is one of the following: leucine (leu), valine (val), isoleucine (ile), tryptophan (trp) alanine (ala), methionine (met), phenylalanine (phe), tyrosine (tyr), histidine (his), and proline (pro). Trimers for use in the invention are selected from leu-leu-gly, leu-leu-ala, leu-leu-val, leu-leu-leu, leu-leu-ile, leu-leu-met, leu-leu-pro, leu-leu-phe, leu-leu-trp, leu-leu-ser, leu-leu-thr, leu-leu-cys, leu-leu-tyr, leu-leu-asp, leu-leu-glu, leu-leu-lys, leu-leu-arg, leu-leu-his, leu-leu-nor, leu-gly-leu, leu-ala-leu, leu-val-leu, leu-ile-leu, leu-met-leu, leu-pro-leu, leu-phe-leu, leu-trp-leu, leu-ser-leu, leu-thr-leu, leu-cys-leu, leu-try-leu, leu-asp-leu, leu-glu-leu, leu-lys-leu, leu-arg-leu, and leu-nor-leu. Particularly preferred peptides are dileucine and trileucine.

[0045] Although less preferred due to their limited solubility in water, additional dispersibility enhancing peptides for use in the invention are 4-mers and 5-mers containing two or more leucine residues. The leucine residues may occupy any position within the peptide, and the remaining (i.e., non-leucyl) amino acids positions are occupied by any amino acid as described above, provided that the resulting 4-mer or 5-mer has a solubility in water of at least about 1 mg/ml. Preferably, the non-leucyl amino acids in a 4-mer or 5-mer are hydrophilic amino acids such as lysine, to thereby increase the solubility of the peptide in water.

[0046] Also preferred are di- and tripeptides having a glass transition temperature greater than about 40 °C.

[0047] Preferred di- and tripeptides for use in the present invention are those peptides that are surface active. As can be seen from the surface tension data in Example 1, dileucine and trileucine are extremely effective, even when present in low concentrations, at significantly depressing the surface tension of water. Moreover, in examining the surface tension results in Table 5 (extrapolated values), it can be seen that dipeptides and tripeptides containing two or more leucines have a much greater surface activity than dipeptides and tripeptides composed of fewer than two leucyl residues. Due to their highly surface active nature, the di- and tripeptides of the invention, when contained in dry powder compositions, tend to concentrate on the surface of the powder particles, thereby imparting to the resulting particles high dispersivities. This feature of the powders, i.e., a surface enriched with the di- or tripeptide, is illustrated by the ESCA data provided in Example 9.

[0048] Surprisingly, the addition of the representative tripeptide, trileucine, to a calcitonin formulation was effective to nearly double the ED value of the resulting powder (Example 4). This result is surprising because calcitonin itself is a surface active protein. Thus, the incorporation of another surface active material such as trileucine was not expected to

significantly improve the dispersivity of the composition. Results in contrast to this expectation indicated that surface activity alone is not sufficient to significantly increase dispersibility, and further demonstrated the unusual and beneficial properties of the leucyl-containing peptides of the invention, particularly in enhancing aerosol performance.

[0049] Generally, the compositions of the invention will contain from 1% to 99% by weight di- or tripeptide, preferably from 2% to 75% by weight di- or tripeptide, and even more preferably from 5% to 50% by weight di- or tripeptide. Typically, the optimal amount of di- or tripeptide is determined experimentally, i.e., by preparing compositions containing varying amounts of di- or tripeptide (ranging from low to high), examining the dispersibilities of the resulting compositions as described herein, and further exploring the range at which optimal aerosol performance is attained. Such methods were employed in several of the Examples (Example 3, Example 4, Example 5, Example 6). Generally, for trileucine containing dry powder formulations, an optimal amount of trileucine appears to be around 22-25% by weight.

C. Additional Carriers and Excipients

[0050] In addition to the active agent and di- or tripeptide, compositions of the invention may optionally include one or more pharmaceutical excipients which are suitable for pulmonary administration. These excipients, if present, are generally present in the composition in amounts ranging from 0.01 % to 95% percent by weight, preferably from 0.5 to 80%, and more preferably from 1 to 60% by weight. Preferably, such excipients will, in part, serve to further improve the features of the active agent composition, e.g., by providing more efficient and reproducible delivery of the active agent, improving the handling characteristics of powders (*e.g.*, flowability and consistency), and/or facilitating manufacturing and filling of unit dosage forms. In particular, excipient materials can often function to further improve the physical and chemical stability of the active agent, minimize the residual moisture content and hinder moisture uptake, and to enhance particle size, degree of aggregation, particle surface properties (*i.e.*, rugosity), ease of inhalation, and the targeting of particles to the lung. The excipient(s) may also serve simply as bulking agents when it is desired to reduce the concentration of active agent in the formulation.

[0051] Pharmaceutical excipients and additives useful in the present composition include but are not limited to amino acids, peptides, proteins, non-biological polymers, biological polymers, carbohydrates (e.g., sugars, derivatized sugars such as alditols, aldonic acids, esterified sugars, and sugar polymers), which may be present singly or in combination. Suitable excipients are those provided in Inhale Therapeutic Systems' International Publication No. WO 96/32096. Also preferred are excipients having glass transition temperatures (Tg), above 35° C, preferably above 40 °C, more preferably above 45° C, most preferably above 55 °C.

[0052] Exemplary protein excipients include albumins such as human serum albumin (HSA), recombinant human albumin (rHA), gelatin, casein, hemoglobin, and the like. Suitable amino acids (outside of the dileucyl-peptides of the invention), which may also function in a buffering capacity, include alanine, glycine, arginine, betaine, histidine, glutamic acid, aspartic acid, cysteine, lysine, leucine, isoleucine, valine, methionine, phenylalanine, aspartame, tyrosine, tryptophan, and the like. Preferred are amino acids and polypeptides that function as dispersing agents. Amino acids falling into this category include hydrophobic amino acids such as leucine, valine, isoleucine, tryptophan, alanine, methionine, phenylalanine, tyrosine, histidine, and proline. Dispersibility- enhancing peptide excipients include dimers, trimers, tetramers, and pentamers comprising one or more hydrophobic amino acid components such as those described above.

[0053] Carbohydrate excipients suitable for use in the invention include, for example, monosaccharides such as fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, maltodextrins, dextrans, starches, and the like; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol sorbitol (glucitol), pyranosyl sorbitol, myoinositol and the like.

[0054] The compositions may also include a buffer or a pH adjusting agent, typically a salt prepared from an organic acid or base. Representative buffers include organic acid salts of citric acid, ascorbic acid, gluconic acid, carbonic acid, tartaric acid, succinic acid, acetic acid, or phthalic acid, Tris, tromethamine hydrochloride, or phosphate buffers.

[0055] The compositions of the invention may also include polymeric excipients/additives, e.g., polyvinylpyrrolidones, derivatized celluloses such as hydroxymethylcellulose, hydroxyethylcellulose, and hydroxypropylmethylcellulose, Ficolls (a polymeric sugar), hydroxyethylstarch, dextrates (e.g., cyclodextrins, such as 2-hydroxypropyl-β-cyclodextrin and sulfobutylether-β-cyclodextrin), polyethylene, glycols, and pectin.

[0056] The compositions may further include flavoring agents, taste-masking agents, inorganic salts (e.g., sodium chloride), antimicrobial agents (e.g., benzalkonium chloride), sweeteners, antioxidants, antistatic agents, surfactants (e.g., polysorbates such as "TWEEN 20" and "TWEEN 80"), sorbitan esters, lipids (e.g., phospholipids such as lecithin and other phosphatidylcholines, phosphatidylethanolamines), fatty acids and fatty esters, steroids (e.g., cholesterol), and chelating agents (e.g., EDTA, zinc and other such suitable cations). Other pharmaceutical excipients and/or additives suitable for use in the compositions according to the invention are listed in "Remington: The Science & Practice of Pharmacy", 19th ed., Williams & Williams, (1995), and in the "Physician's Desk Reference", 52nd ed., Medical Economics, Montvale, NJ (1998).

III. Formulation Types

[0057] The compositions described herein may be in powdered form or may be flowable liquids. Liquid formulations are preferably solutions in which the active drug is dissolved in a solvent (e.g., water, ethanol, ethanol-water, saline) and less preferably are colloidal suspensions. The liquid formulation may also be a solution or suspension of the active agent in a low boiling point propellant.

[0058] Liquid formulations containing the disclosed dileucyl-containing peptides are also highly dispersible, possessing high ED values.

IV. Preparing Drv Powders

[0059] Dry powder formulations are preferably prepared by spray drying. Spray drying of the formulations is carried out, for example, as described generally in the "Spray Drying Handbook", 5th ed., K. Masters, John Wiley & Sons, Inc., NY, (1991), and in Platz, R., *et al*., International Patent Publication No. WO 97/41833 (1997).

[0060] Active agents having a solubility in water of at least about 0.10 mg/ml (e.g., peptides, proteins, nucleotides and the like) can be sprayed dried from an aqueous solution. Utilizing this approach, the active agent is first dissolved in water, optionally containing a physiologically acceptable buffer. The pH range of active agent-containing solutions is generally between about 4 and 11, with nearer neutral pHs being preferred, since such pHs may aid in maintaining the physiological compatibility of the powder after dissolution of powder within the lung. The aqueous formulation may optionally contain additional water-miscible solvents, such as acetone, alcohols and the like. Representative alcohols are lower alcohols such as methanol, ethanol, propanol, isopropanol, and the like. The pre-spray dried solutions will generally contain solids dissolved at a concentration from 0.01% (weight/volume) to about 20% (weight/volume), usually from 0.1% to 3% (weight/volume).

[0061] The solutions are then spray dried in a conventional spray drier, such as those available from commercial suppliers such as Niro A/S (Denmark), Buchi (Switzerland) and the like, resulting in a dispersible, dry powder. Optimal conditions for spray drying the solutions will vary depending upon the formulation components, and are generally determined experimentally. The gas used to spray dry the material is typically air, although inert gases such as nitrogen or argon are also suitable. Moreover, the temperature of both the inlet and outlet of the gas used to dry the sprayed material is such that it does not cause decomposition of the active agent in the sprayed material. Such temperatures are typically determined experimentally, although generally, the inlet temperature will range from 50° C to 200° C while the outlet temperature will range from 30° C to 150° C.

[0062] Variations of the above are utilized for spray-drying formulations where the active agent is a hydrophobic drug. One such process is described in Gordon, M.S., Lord, J.D., U.S. Patent No. 5,985,248, assigned to Inhale Therapeutic Systems. In X this method, a hydrophobic drug is dissolved in an organic solvent or co-solvent system, and the hydrophilic components (e.g., the leucyl-containing peptides and optional other excipients) are at least partially dissolved in the same organic solvent or co-solvent system. The resulting solution is then spray-dried to form particles. Typically, the solubility of the active agent and the hydrophilic component will govern the selection of the organic solvent system. The organic solvent is selected to provide a solubility for the hydrophilic component of at least 1 mg/ml, and preferably at least 5 mg/ml, and a solubility for the hydrophobic drug of at least 0.01 mg/ml, preferably at least 0.05 mg/ml.

[0063] Alternatively, the composition may be prepared by spray-drying a suspension, as described in Gordon, M.S., U.S. Patent No. 5,976,574, assigned to Inhale Therapeutic Systems. In this method, the hydrophobic drug is dissolved in an organic solvent, e.g., methanol, ethanol, isopropanol, acetone, heptane, hexane, chloroform, ether, followed by suspension of the hydrophilic excipient in the organic solvent to form a suspension. The suspension is then spray-dried to form particles. Preferred solvents, for both of the above spray-drying methods include alcohols, ethers, ketones, hydrocarbons, polar aprotic solvents, and mixtures thereof.

[0064] The dry powders of the invention may also be prepared by combining aqueous solutions or suspensions of the formulation components and spray-drying them simultaneously in a spray-dryer, as described in Gordon, M., U.S. Patent No. 6,001,336, assigned to Inhale Therapeutic Systems. Alternatively, the dry powders may be prepared by preparing an aqueous solution of a hydrophilic excipient or additive, preparing an organic solution of a hydrophobic drug, and spray drying the aqueous solution and the organic solution simultaneously through a nozzle, e.g., a coaxial nozzle, to form a dry powder, as described in Gordon, M., et al, International Publication Number WO 98/29096.

[0065] Alternatively, powders may be prepared by lyophilization, vacuum drying, spray freeze drying, super critical fluid processing, air drying, or other forms of evaporative drying. In some instances, it may be desirable to provide the dry powder formulation in a form that possesses improved handling/processing characteristics, e.g., reduced static, better flowability, low caking, and the like, by preparing compositions composed of fine particle aggregates, that is, aggregates or agglomerates of the above-described dry powder particles, where the aggregates are readily broken back down to the fine powder components for pulmonary delivery, as described, e.g., in Johnson, K., et al., U.S. Patent No. 5,654,007, 1997.

[0066] In another approach, dry powders may be prepared by agglomerating the powder components, sieving the materials to obtain agglomerates, spheronizing to provide a more spherical agglomerate, and sizing to obtain a uniformly-sized product, as described, e.g., and in Ahlneck, C., et al., International PCT Publication No. WO 95/09616, 1995.

[0067] Dry powders may also be prepared by blending, grinding, sieving or jet milling formulation components in dry powder form.

[0068] Once formed, the dry powder compositions are preferably maintained under dry (i.e., relatively low humidity) conditions during manufacture, processing, and storage. Irrespective of the drying process employed, the process will preferably result in respirable, highly dispersible particles comprising an active agent and a dileucyl-containing dimer or trimer.

V. Features of Dry Powder Formulations

[0069] Powders of the invention are further characterized by several features, most notably, (i) consistently high dispersivities, which are maintained, even upon storage (Example 8), (ii) small aerodynamic particles sizes (MMADs), (iii) improved fine particle dose values, i.e., powders having a higher percentage of particles sized less than 3.3 microns MMAD, all of which contribute to the improved ability of the powder to penetrate to the tissues of the lower respiratory tract (i.e., the alveoli) for either localized or systemic treatment. These physical characteristics of the di-leucyl peptide-containing dry powders, to be described more fully below, are important in maximizing the efficiency of aerosolized delivery of such powders to the deep lung.

[0070] Dry powders of the invention are composed of aerosolizable particles effective to penetrate into the lungs. The particles of the invention have a mass median diameter (MMD) of less than 20 μm, preferably less than 10 μm, more preferably less than 7.5 μm, and most preferably less than 4 μm, and usually are in the range of 0.1 μm to 5 μm in diameter. Preferred powders are composed of particles having an MMD from 0.2 to 4.0 μm. In some cases, the powder will also contain non-respirable carrier particles such as lactose, where the non-respirable particles are typically greater than about 40 microns in size.

[0071] The powders of the invention are further characterized by an aerosol particle size distribution less than about 10 μm mass median aerodynamic diameter (MMAD), and preferably less than 4.0 μm. The mass median aerodynamic diameters of the powders will characteristically range from 0.1 - 10 μm, preferably from 0.2 - 5.0 μm MMAD, more preferably from 1.0 - 4.0 μm MMAD, and even more preferably from 1.5 to 3.5 μm. Illustrative MMAD values for exemplary di-leucyl-peptide-containing powder compositions are provided in Examples 2, 3, 4, 5, and 6. Several of these examples demonstrate an improvement in aerosol particle size distribution achieved upon incorporation of a di-leucyl di- or tripeptide into the formulation.

[0072] The powders of the invention may further be characterized by their densities. The powder will generally possess a bulk density from 0.1 to 10 g/cubic centimeter, preferably from 0.1-2 g/cubic centimeter, and more preferably from 0.15-1.5 g/cubic centimeter.

[0073] The powders will generally have a moisture content below 20% by weight, usually below 10% by weight, and preferably below 6% by weight. Such low moisture-containing solids tend to exhibit a greater stability upon packaging and storage.

[0074] One of the most striking features of the compositions of the invention is their dispersibility, as indicated by the ED value. The presence of the di-leucyl peptide in the formulations is effective to provide formulations having significantly improved dispersibilities. Generally, the emitted dose (ED) of these powders is greater than 30%, and usually greater than 40%. More preferably, the ED of the powders of the invention is greater than 50%, and is often greater than 55%. In fact, in looking at the Examples, di-leucyl-peptide containing powders typically possess optimized ED values as high as 80% or above. Moreover, the Examples further illustrate that the incorporation of a di-leucyl di- or tripeptide into a variety of active agent formulations was effective, in all cases, to increase the ED value of the resultant compositions, and in some instances, as much as doubling its value. Moreover, this effect was observed for both protein and small molecule active agent powders.

[0075] An additional measure for characterizing the overall aerosol performance of a dry powder is the fine particle dose (FPD), which describes the percentage of powder having an aerodynamic diameter less than 3.3 microns. The powders of the invention are particularly well suited for pulmonary delivery, and possess FPD values ranging from 35%-85%. Such powders contain at least 35 percent of aerosol particle sizes below 3.3 μm to 0.5 μm and are thus extremely effective when delivered in aerosolized form, in reaching the regions of the lung, including the alveoli.

[0076] The compositions described herein also possess good stability with respect to both chemical stability and physical stability, i.e., aerosol performance, over time (Example 8). Generally, with respect to chemical stability, the active agent contained in the formulation will degrade by no more than 10% over a time course of three months, preferably by no more than 7%, and more preferably by no more than 5%, upon storage of the composition under ambient conditions. As illustrated by the exemplary PTH formulation in Example 8, storage under accelerated stability conditions (40 °C, ambient humidity) for a period of 3 months (12 weeks) resulted in the degradation of only 2.3% protein (from an initial

value of 97.1% purity to 94.8% purity). Since accelerated temperatures result in an increase in reaction rate, one can conclude that storage of the same composition under ambient conditions would result in a degradation rate less than 2.3%, further pointing to the chemical stability of the present compositions.

[0077] With respect to aerosol performance, compositions of the invention are generally characterized by a drop in emitted dose of no more than about 20%, preferably no more than 15%, and more preferably by no more than 10%, when stored under ambient conditions for a period of three months. In looking at the results in Example 8, an exemplary PTH-trileucine formulation exhibited essentially no change, and in particular, no diminishment, in aerosol properties (MMAD, FPD, ED) upon storage under accelerated stability conditions (40 °C, ambient humidity)

[0078] Another preferred feature of particulate compositions of the invention is an enrichment of the di-leucyl di- or tripeptide on the surface of the particles, as indicated by the results in Example 9.

[0079] The improvement in aerosol properties discovered for di-leucyl di- and tripeptide-containing composition (i.e., greatly enhanced dispersibilities, reduced fine particle dose values, smaller aerodynamic diameters), can result in several related advantages, such as: (i) reducing costly drug loses to the inhalation device, since more powder is aerosolized and is therefore available for inhalation by a subject; (ii) reducing the amount of dry powder required per unit dose, due to the high efficiency of aerosolization of powder, (iii) reducing the number of inhalations per day by increasing the amount of aerosolized drug reaching the lungs of a subject.

VI. Administration of the Composition

[0080] The formulations described herein may be delivered using any suitable dry powder inhaler (DPI), *i.e.*, an inhaler device that utilizes the patient's inhaled breath as a vehicle to transport the dry powder drug to the lungs. Preferred are Inhale Therapeutic Systems' dry powder inhalation devices as described in Patton, J.S., et al., U.S. Patent No. 5,458,135 (1995); Smith, A.E., et al., U.S. Patent No. 5,740,794 (1998); and in Smith, A.E., et. al., U.S. Patent No. 5,785,049 (1998).

[0081] When administered using a device of this type, the powder is contained in a receptacle having a puncturable lid or other access surface, preferably a blister package or cartridge, where the receptable may contain a single dosage unit or multiple dosage units. Convenient methods for filling large numbers of cavities (i.e., unit dose packages) with metered doses of dry powder medicament are described, e.g., in Parks, D.J., et al., WO 97/41031 (1997).

[0082] Also suitable for delivering the powders described herein are dry powder inhalers of the type described, for example, in Cocozza, S., et al., U.S. Patent No. 3,906,950 (1974), and in Cocozza, S., et al., U.S. Patent No. 4,013,075 (1997), wherein a premeasured dose of dry powder for delivery to a subject is contained within a hard gelatin capsule.

[0083] Other dry powder dispersion devices for pulmonarily administering dry powders include those described, for example, in Newell, R.E., *et al*, European Patent No. EP 129985 (1988); in Hodson, P.D., *et al.*, European Patent No. EP 472598 (1996); in Cocozza, S., *et al.*, European Patent No. EP 467172 (1994), and in Lloyd, L.J. et al., U.S. Patent No. 5,522,385 (1996). Also suitable for delivering the dry powders of the invention are inhalation devices such as the Astra-Draco "TURBUHALER". This type of device is described in detail in Virtanen, R., U.S. Patent No. 4,668,281 (1987); in Wetterlin, K., et al U.S. Patent No. 4,667,668 (1987); and in Wetterlin, K., et al. U.S. Patent No. 4,805,811 (1989). Other suitable devices include dry powder inhalers such as the Rotahaler® (Glaxo), Discus® (Glaxo), Spiros™ inhaler (Dura Pharmaceuticals), and the Spinhaler® (Fisons). Also suitable are devices which employ the use of a piston to provide air for either entraining powdered medicament, lifting medicament from a carrier screen by passing air through the screen, or mixing air with powder medicament in a mixing chamber with subsequent introduction of the powder to the patient through the mouthpiece of the device, such as described in Mulhauser, P., et al, U.S. Patent No. 5,388,572 (1997).

[0084] Dry powders may also be delivered using a pressurized, metered dose inhaler (MDI), e.g., the Ventolin® metered dose inhaler, containing a solution or suspension of drug in a pharmaceutically inert liquid propellant, e.g., a chlorofluorocarbon or fluorocarbon, as described in Laube, et al., U.S. Patent No. 5,320,094 (1994), and in Rubsamen, R.M., et al, U.S. Patent No. 5,672,581 (1994), Alternatively, the powders described herein may be dissolved or suspended in a solvent, e.g., water, ethanol, or saline, and administered by nebulization. Nebulizers for delivering an aerosolized solution include the AERx™ (Aradigm), the Ultravent® (Mallinkrodt), and the Acorn II® (Marquest Medical Products).

[0085] Prior to use, dry powders are generally stored under ambient conditions, and preferably are stored at temperatures at or below 25°C, and relative humidities (RH) ranging from 30 to 60%. More preferred relative humidity conditions, e.g., less than 30%, may be achieved by the incorporation of a dessicating agent in the secondary packaging of the dosage form.

VII. Utility

[0086] The compositions of the invention are useful, when administered pulmonarily in a therapeutically effective amount to a mammalian subject, for treating or preventing any condition responsive to the administration of an active agent as described in section II.A above.

[0087] The following examples are illustrative of the present invention, and are not to be construed as limiting the scope of the invention. Variations and equivalents of the examples will be apparent to those of skill in the art in light of the present disclosure, the drawings and the claims herein.

Examples

Materials and Methods

A. Materiais.

[0088] Ciprofloxacin Hydrochloride (Neuland Laboratories, India).
Gentamicin Sulfate (H&A (Canada) Industrial)
Netilmicin Sulfate (Scientific Instruments And Technology)
L-Leucine (Aldrich, St. Louis, MO)
Hydrochloric Acid (J. T. Baker, Phillipsburg, N.J.)
Sodium Hydroxide 0.1N Volumetric Solution (J. T. Baker, Phillipsburg, N.J.)
Ethanol, 200 proof (USP/NF, Spectrum Chemical Mfg. Corp., New Brunswick, N.J.)
Methanol (HPLC grade, EM Industries, Gibbstown, N.J.)
S. calcitonin (Bachem California Inc, USA, Torrance, CA).
Trileucine (Bachem California Ine, USA, Torrance, CA).
Other amino acids used in surface tension experiments were obtained from Sigma, St. Louis, MO.

B. Methods.

Particle size measurements (Horiba)

[0089] Mass median diameters (MMD) of the powders were measured using a Horiba CAPA-700 particle size analyzer (Horiba Instruments Inc., Irvine, CA). Measurements were based upon centrifugal sedimentation of dispersed particles in suspending medium. Mass median diameter, which is based on the particle's Stokes' diameter, was calculated using the particle density and the density and viscosity of the suspending medium.

[0090] The density of the powder was set as 1.5 g/cm^3 for all powders. (This nominal value was used for all powders analyzed and is within a range that is typical for spray dried powders). Particle size measurements were conducted with about 5 - 10 mg powder suspended in ml Sedisperse A-11 (Micromeritics, Norcross, GA) and dispersed by sonication for 10 minutes. The range over which particle size data was gathered was set to 0.4 to 10.0 μm.

Aerodynamic Particle Size Measurements

[0091] Andersen Cascade Impactor. An Andersen cascade impactor (a sieve-like apparatus with a series of stages that capture particles on plates by inertial impaction according to their size) was used to determine the MMAD and particle size distribution of aerosolized powder formulations in an air stream. The plates were weighed before and after testing and the mass of powder deposited on the plate of each stage was determined. Unless otherwise indicated, studies were undertaken using a traditional Andersen cascade impactor having eight stages (from top to bottom stages 0 to 7) with cut-off sizes ranging from 9.0 to 0.4 μm, and a final filter stage that traps particles < 0.4 μm when operated at a flow rate of 28.3 L/min. The device test set-up was similar to the ED test, except that the cascade impactor and a USP (United States Pharmacopeia) throat (USP 23, chapter <601>) were attached to the device mouthpiece rather than to a filter. Multiple dispersions were typically conducted for each cascade impaction run to achieve gravimetrically accurate data.

[0092] Andersen Short Stack (SS) Method. In the SS method, the order in which the stages were placed was altered from the conventional Andersen cascade impactor set-up as described above. From the top, stage 0 was utilized for inlet cone attachment to connect the throat. Stage 3 was positioned next, beneath stage 0, followed by the filter stage (stage F). The powder-containing airstream passes only through stages 0 and 3; air (but not powder) flows through the other stages, which are placed under stage F to hold the remainder of the assembly in place. A pre-weighed filter was placed on stage F and captured particles < 3.3 μm. A second filter was placed on an inverted plate under stage 3, and captured particles > 3.3 μm. For the studies described herein, one BP (blister pack) containing 2 mg of powder composition was dispersed in an aerosol delivery device and a vacuum was pulled at 28.3 L/min as per USP methodology. This process was then repeated two times for a target mass of 6 mg per run. The filters were then removed and weighed to determine the amount of powder deposited.

Example 1

Surface Activity of Di- and Tripeptides

[0093] The surface tension of several representative dipeptides, tripeptides, and proteins was measured at 25 °C and 45 °C to provide an indication of their relative surface activities. Surface tension measurements were carried out using a Kruss Processor Tensiometer-K12 with the Wilhelmy-method (Plate method).

[0094] Solutions were prepared by dissolving either 0.05%, 0.2%, or 0.6% peptide/protein (by weight) along with an appropriate amount of raffinose by weight to provide final solutions having a 1.0% by weight solids content. Surface tension measurements at 25 °C and 45 °C were then obtained for the test solutions at three different time points (49 seconds, 100 seconds and 194 seconds). The results are shown in Tables 1-5 below.

[0095] Highly surface active peptides and proteins are those that are effective to lower the surface tension of water from its control value(s). As can be seen in Tables 1-4, raffinose (which was added to each of the solutions to bring the overall solids content to 1.0%) is non-surface active, and thus does not impact the surface tension results obtained for each of the peptides/proteins.

[0096] In looking at the results below, it can be seen that highly surface active peptides include the peptides, dileucine and trileucine. These peptides were as effective as the highly surface active protein, salmon calcitonin, at significantly lowering the surface tension of water. Trileucine was effective at lowering the surface tension of water to a greater extent at higher concentrations (see, for example, data for 0.05%, 0.2% and 0.6% by weight tri-leucine). In comparison to trileucine and dileucine, the dimer of isoleucine and the dimer and trimer of valine were not particularly effective at lowering the surface tension of water.

[0097] This method can be used to identify additional surface active di- and tri-peptides suitable for use in the dry powders of the invention.

Table 1. Surface Tension Measurements

SAMPLE	ST.mN/m	time.s	ST.mN/m	time.s	St.mN/m	time.s
water blank-1	72.6	49	72.6	100	72.6	194
water blank-2	72.5	49	72.5	100	72.4	194
water blank-3	72.5	49	72.4	100	72.4	194
1% raffinose-1	72	49	72	100	72	194
1% raffinose-2	72	49	72	100	72	194
1% raffinose-3	72	49	72	100	72	194
0.2% tri-alanine-1	72.4	49	72.4	100	72.3	194
0.2% tri-alanine-2	72.2	49	72.2	100	72.2	194
0.2% tri-alanine-3	72.3	49	72.2	100	72.2	194
0.2% tri-glutamate-1	72.1	49	72.1	100	72.1	194
0.2% tri-glutamate-2	72.4	49	72.4	100	72.4	194
0.2% tri-glutamate-3	72.4	49	72.4	100	72.3	194
0.2% di-alanine-1	72	49	72	100	71.9	194
0.2% di-alanine-2	72	49	71.9	100	71.9	194
0.2% di-alanine-3	72.1	49	72.1	100	72.1	194
0.2% di-leucine-1	58.4	49	58.1	100	57.9	194

(continued)

SAMPLE	ST.mN/m	time.s	ST.mN/m	time.s	St.mN/m	time.s
0.2% di-leucine-2	58.7	49	58.3	100	58.2	194
0.2% di-leucine-3	60.1	49	59.8	100	59.7	194
0.2% tri-leucine-1	51	49	50.9	100	50.9	194
0.2% tri-leucine-2	51	49	50.8	100	50.7	194
0.2% tri-leucine-3	51	49	50.8	100	50.7	194
0.2% sal. Calcitonin-1	48.7	49	48.6	100	48.5	194
0.2% sal.calcitonin-2	48.4	49	48.4	100	48.4	194
0.2% sal.calcitonin-3	48.4	49	48.4	100	48.4	194
Measurements conducted at 25 °C. The 0.2% (wt/vol) solutions additionally contain 5 raffinose to form solutions having a total solids content of 1% (wt/vol).						

Table 2. Surface Tension Measurements

SAMPLE	ST.mN/m	time.s	ST.mN/m	time.s	St.mN/m	time.s
water blank-1	72	49	71.8	100	71.7	194
water blank-2	72.2	49	72.2	100	72.2	194
water blank-3	71.5	49	71.6	100	71.6	194
0.2% di-isoleucine-1	67.6	49	67.2	100	67	194
0.2% di-isoleucine-2	68	49	67.8	100	67.6	194
0.2% di-isoleucine-3	67.7	49	71.6	100	71.6	194
0.2% di-valine-1	71.7	49	71.6	100	71.6	194
0.2% di-valine-2	71.6	49	71.6	100	71.6	194
0.2% di-valine-3	71.7	49	71.6	100	71.6	194
0.2% tri-valine-1	68.8	49	68.8	100	68.8	194
0.2% tri-valine-2	68.8	49	68.7	100	68.7	194
0.2% tri-valine-3	68.7	49	68.7	100	68.7	194
Surface tension measurements conducted at 25 °C. Solutions contained 0.20% (wt/vol) of one of: di-isoleucine, di-valine, or tri-valine and 0.80% (wt/vol) raffinose.						

Table 3. Surface Tension Measurements

SAMPLE	ST.mN/m	time.s	ST.mN/m	time.s	St.mN/m	time.s
1% raffinose (pH4)-1	71.4	49	71.4	100	71.4	194
1% raffinose (PH4)-2	71.1	49	71.1	100	71.1	194
1% raffinose (pH4)-3	71.1	49	71.1	100	71.1	194
1% raffinose(pH7)-1	71.1	49	71.1	100	71.1	194
1% raffinose (pH7)-2	71.1	49	71.1	100	71.1	194
1% raffinose (pH7)-3	71.1	49	71.1	100	71.1	194
water blank-1	72.1	49	72	100	72	194
water blank-2	72.2	49	72.1	100	72	194
water blank-3	72.2	49	72.1	100	72	194
0.05% leu3(pH4)-1	59.9	49	59.8	100	59.7	194
0.05% leu3(pH4)-2	60.4	49	60.3	100	60.2	194
0.05% leu3(pH4)-3	60.4	49	60.3	100	60.2	194
0.2% leu3(pH4)-1	51.4	49	51.2	100	51.1	194
0.2% leu3(pH4)-2	51.4	49	51.3	100	51.2	194
0.2% leu3(pH4)-3	51.4	49	51.2	100	51.1	194
0.6% leu3(pH4)-1	44.2	49	44.1	100	44	194
0.6% leu3(pH4)-2	44.3	49	44.2	100	44.2	194
0.6% leu3(pH4)-3	44.2	49	44.2	100	44.1	194
0.05% leu3(pH7)-1	60.1	49	59.8	100	59.7	194
0.05% leu3(pH7)-2	60	49	59.8	100	59.7	194
0.05% leu3(pH7)-3	60.2	49	60	100	59.8	194
0.2% leu3(pH7)-1	51	49	50.8	100	50.7	194
0.2% leu3(pH7)-2	50.9	49	50.7	100	50.6	194
0.2% leu3(pH7)-3	50.7	49	50.5	100	50.4	194
0.6% leu3(pH7)-1	43.7	49	43.7	100	43.6	194
0.6% leu3(pH7)-2	43.8	49	43.7	100	43.7	194
0.6% leu3(pH7)-3	43.8	49	43.7	100	43.7	194
water blank-5	71.7	49	71.7	100	71.6	194
water blank-6	72.2	49	72.1	100	72.1	194

(continued)

SAMPLE	ST.mN/m	time.s	ST.mN/m	time.s	St.mN/m	time.s
Surface tension measurements measured at 25 °C. The trileucine formulations also contain raffinose to provide solutions having a total solids content of 1% (wt/vol).						

Table 4. Surface Tension Measurements

SAMPLE	ST.mN/m	time.s	ST.mN/m	time.s	St.mN/m	time.s
water blank-5	69.2	49	69.2	100	69.2	194
1% raffinose(pH4)-1	67.9	49	68	100	68	194
1% raffinose (pH4)-2	68.2	49	68.2	100	68.2	194
1% raffinose (pH4)-3	68	49	68	100	68.1	194
1% raffinose (pH7)-1	68.3	49	68.3	100	68.3	194
1% raffinose (pH7)-2	68.4	49	68.4	100	68.4	194
1% raffinose (pH7)-3	68.4	49	68.4	100	68.4	194
Leu3 formulations contain raffinose to make 1% total solids content						
0.05% leu3(pH4)-1	57.1	49	57	100	57	194
0.05% leu3(pH4)-2	58.1	49	57.9	100	57.8	194
0.05% leu3(pH4)-3	58	49	57.8	100	57.8	194
0.2% leu3(pH4)-1	47.9	49	47.5	100	47.4	194
0.2% leu3(pH4)-2	47.2	49	47.2	100	47.3	194
0.2% leu3(pH4)-3	47.9	49	47.3	100	47.1	194
0.6% leu3(pH4)-1	40.9	49	40.9	100	40.8	194
0.6% leu3(pH4)-2	41.1	49	41	100	40.9	194
0.6% leu3(pH4)-3	41.1	49	41	100	40.8	194
pH						
0.05% leu3(pH7)-1	58.5	49	58.4	100	58.4	194
0.05% leu3(pH7)-2	58.2	49	58.2	100	58.1	194
0.05% leu3(pH7)-3	58.2	49	58.1	100	58.1	194
0.2% leu3(pH7)-1	58.5	49	58.4	100	58.4	194
0.2% leu3(pH7)-2	58.2	49	58.2	100	58.1	194
0.2% leu3(pH7)-3	58.2	49	58.1	100	58.1	194

(continued)

SAMPLE	ST.mN/m	time.s	ST.mN/m	time.s	St.mN/m	time.s
Surface tension measurements taken at 45°C. Tri-leucine-containing formulations also contain raffinose to provide a solution having a total solids content of 1%. Additional surface tension measurements were obtained to determine dimers and trimers for use in the invention (i.e.. surface active dimers and trimers).						

Table 5. Surface Tension of Representative Dimers and Trimers

		25 °C		45 °C		25 °C	45 °C
		Actual		Actual		Extrapolated Values	Extrapolated Values
SAMPLE	concentration mg/ml	MEAN	SD	MEAN	SD	at 2 mg/ml	at 2 mg/ml
Dimers							
Leu-2	13.60	46.6	0.6	42.7	0.3	60.4	52.4
	4.53	54.9	0.5	48.2	0.1		
	1.51	61.5	0.6	53.2	0.1		
Leu-Val *	8.80	59.1	0.2	55.7	0.3	67.2	62.3
	2.93	65	0.2	60.4	0.0		
	0.98	69.2	0.4	64.2	0.2		
Leu-Tvr *	6.40	62.2	0.1	59.5	0.3	68.3	67.3
	2.13	68.0	0.1	65.6	0.3		
	0.71	71.5	0	68.0	0.1		
Val-Leu *	7.80	68	0	63.5	0.2	69.8	65.3
	2.60	69.5	0.1	65.0	0.2		
	0.87	70	0.5	65.5	0.0		
Val-Ile *	10.00	66.1	0	61.9	0.2	70.3	65.8
	3.33	70.1	0.3	65.3	0.1		
	1.11	71.6	0.2	66.3	0.2		
Leu *	TBD	56.7	0.3	54.7	0.2		
		66.3	0.2	61.4	0.2		
Trimers		70.8	0.2	64.2	0.0		
Leu-Tyr-Leu	2.90	44.7	0.1	40.8	0.0	47.9	44.7
	0.97	51.6	0.1	49.1	0.1		
	0.32	58.4	0.1	55.4	0.3		
Leu-Phe-Leu	6.10	41.5	0.2	39.3	0.0	48.3	46.2
	2.03	48.3	0	46.2	0.1		
	0.68	54.7	0.1	53.6	0.1		
Leu-3	6.10	42.4	0	38.9	0.2	49.7	46.3
	2.03	49.7	0	46.3	0.3		

(continued)

		25 °C		45 °C		25 °C	45 °C
		Actual		Actual		Extrapolated Values	Extrapolated Values
SAMPLE	concentration mg/ml	MEAN	SD	MEAN	SD	at 2 mg/ml	at 2 mg/ml
Dimers							
	0.68	56.9	0	52.8	0.6		
Leu-Leu-Ala	6.80	39.9	0.5	48.4	0.2	46.6	49.8
	2.27	43.5	0.8	48.2	4.3		
	0.76	60.7	0.4	58.3	0.5		
Ala-Val-Leu *	8.70	55.7	0.2	53.8	0.0	65	58.9
	2.90	62.8	0.5	57.7	0.2		
	0.97	67.5	0.5	60.3	0.1		
* Reference samples							

[0098] As can be seen from the above, surface active dimers and trimers are more effective when present at higher concentrations at lowering the surface tension of water. As an example, at a concentration of 1.20 mg/ml, the presence of trileucine was effective to lower the surface tension of water from about 72 mN/m to 42 mN/s, while at a concentration of 0.68 mg/ml, trileucine was effective at lowering the surface tension of water to about 57 mN/m

[0099] To normalize for concentration effects, surface tension values were extrapolated to solutions having a concentration of 2 mg/ml (Table 5, columns 7 and 8). Looking first at the dimers, dileucine was more effective than any of the other dimers examined in reducing the surface tension of water. Looking at data for the trimers, leu-tyr-leu is the most surface active of the trimers. Trimers containing, in addition to two leucyl residues, a hydrophobic amino acid such as tyrosine, phenylalanine, leucine, or alanine, are more surface active than trimers containing fewer than two leucyl residues.

[0100] In summary, dimers and trimers containing two or more leucines were effective at significantly lowering the surface tension of water (e.g., leu-try-ala, leu-phe-leu, leu-leu-leu, leu-leu-ala, and the like), and are preferred for use in the compositions of the invention.

Example 2

Aerosol Properties of a Parathyroid Hormone (PTH)-Trileucine Dry Powder

[0101] Dry powders containing an illustrative active protein, parathyroid hormone, in combination with either leucine or tri-leucine, were prepared. Also prepared was a dry powder absent either leucine or trileucine, to demonstrate the notable improvement in aerosol properties upon addition of trileucine.

[0102] Representative PTH powders were prepared as follows.

A. Solution Formulation Preparation

[0103] Aqueous formulation solutions were prepared at a total solids content of 1% (w/v). The pH of each solution was determined, and solutions were then spray-dried. Table 6 lists the compositions of all pre-spray-dried PTH solutions.

B. Powder Processing: Spray Drying

[0104] Powders were produced by spray drying aqueous solutions of PTH as described in A. above using a Buchi 190 mini spray dryer (Buchi Labortechnik AG, Meierseggstrasse, Switzerland) equipped with a customized nozzle (Platz, R., *et al.*, Inhale Therapeutic Systems' International Patent Publication No. WO 97/41833, Nov. 13, 1997) and cyclone. High collection efficiencies (yields), usually between about 50-80%, were attained.

Table 6. PTH Dry Powder Compositions

Lot No.	Composition	Emitted Dose, % mean n=10	RSD, %	MMAD (μm)	FPD
R97190 *	30% PTH 70% mannitol	62	4	-	-
*	30% PTH 70% raffinose	66	9	-	-
R97191 *	75% PTH 25% mannitol	51	3	-	-
*	30% PTH 70% leu	78	-	2.43	0.58
	30% PTH 70% tri-leu	83	-	2.63	0.45
* Reference Compositions					

[0105] In looking at the results in Table 6 (and in other tables as well), it can be seen that the addition of trileucine is effective to significantly improve the aerosol performance of the resulting powder. The aerosol performance of a PTH dry powder, as indicated by its ED value, was unexpectedly increased from 51-62% to 83% by the addition of tri-leucine to the formulation. These data illustrate a tremendous improvement in emitted dose, achieved simply by addition of the exemplary surface active tripeptide, tri-leucine to the formulation. Surprisingly, even upon correcting on a mole-to-mole basis for the number of leucine amino acids contained in trileucine (3 moles leu per mole of trileucine), trileucine is more effective than leucine, on a per weight basis, at increasing the dispersivity of dry powder compositions for delivery to the lung.

Example 3

Aerosol Properties of Albuterol-Trileucine Dry Powders

[0106] Dry powders containing the small molecule, albuterol, were prepared to examine the effects of trileucine on the dispersivity/aerosol properties of dry powders containing a non-proteinaceous active agent.

A. Solution Formulation Preparation

[0107] Formulation solutions were prepared at a total solids content of 1% (w/v). For low solids-containing solutions, raffinose was added to bring the total solids content to the above value. Table 7 lists the compositions of all pre-spray dried solutions.

37. Powder Processing: Spray Drying

[0108] Powders were produced by spray drying aqueous solutions of albuterol, surface active di- or tri-peptide, and/or other excipient(s) using a Buchi 190 mini spray dryer (Buchi Labortechnik AG, Meierseggstrasse, Switzerland) as described in Example 2 above. Characteristics of the resultant powders are provided in Tables 7 and 8 below.

Table 7. Albuterol Dry Powders

Formulation	Emitted Dose, %	Tg, °C
2% albulerol * 98% raffinose	31	102.2
2% albuterol * 5% leucine raffinose	31	88.57

(continued)

Formulation	Emitted Dose, %	Tg, °C
2% albuterol * 20% leucine raffinose	34	93.1
2% albuterol * 60% leucine raffinose	74	96.6
2% albuterol 5% trileucine raffinose	62	85.3
2% albuterol 20% trilcucine raffinose	78	95.9
2% albuterol 60% trileucine raffinose	82	88.6
* Reference Formulations		

Table 8. Additional Aerosol Properties of Albuterol Dry Powders

Formulation	FPD	MMAD, microns
2% albuterol * 60% leucine raffinose	0.56	2.43
2% albuterol 20% tri-leucine raffinose	0.59	2.43
* Reference Formulation		

[0109] As can be seen from the results provided above, the addition of trileucine increased the emitted dose of albuterol dry powders from about 30% to about 80% - an improvement in dispersivity of nearly three-fold! Thus, the addition of a surface active di- or tri-peptide to an active agent dry powder can by greatly improving the powder's dispersivity, (i) reduce costly drug loses to the inhalation device, (ii) reduce the number of required inhalations per day by increasing the amount of aerosolized drug reaching the alveoli of a patient, (iii) reduce the amount of dry powder per unit dose, due to the high efficiency of aerosolization of dry powder, and (iv) increase the ease of manufacturing unit dosage forms of powdered drug, due to increased flowability of powder.

[0110] Additionally, the addition of 60% by weight leucine was required to achieve the same level of dispersivity achieved by the addition of only 20% by weight tri-leucine. Thus, tri-leucine is much more effective than leucine in improving the aerosol performance of dry powders. Moreover, a maximum in aerosol performance is typically achieved by the addition of only from about 5-25% (wt) trileucine; quantities greater than that typically provide only incremental improvements in dispersivity.

[0111] The dispersibility-enhancing effects of tri-leucine, and other surface active di- and tri-peptides, appear to be general, and extend to not only protein powders, but to powdered formulations of a wide variety of active agents (e.g., small molecules, hormones, antibiotics, and the like), as illustrated by the Examples provided herein.

Example 4

Aerosol Properties of Salmon Calcitonin-Trileucine Dry Powders

[0112] The effects of trileucine on the aerosol performance of dry powders containing salmon calcitonin, a hormone with a molecular weight of approximately 4500 daltons, were examined.

[0113] Although salmon calcitonin is a highly surface active protein, spray-dried powders containing 5% (wt) salmon calcitonin and 95% (wt) raffinose exhibited relatively low emitted dose values (of approximately 50%). In efforts to further explore the broad applicability of adding surface active di- and tri-peptides to powder formulations to increase their dispersivity, tri-leucine was added to salmon calcitonin-containing formulations to examine its impact on the resulting powders. The ability of tri-leucine to improve the dispersibility of salmon calcitonin containing dry powders was compared to the amino acid, leucine.

[0114] Powders having the compositions indicated below were prepared as described in Examples 2 and 3 above.

Table 9. S. Calcitonin Dry Powders

Formulation	Emitted Dose	FPD	Tg, °C
5% s. Calcitonin * 95% raffinose	48	0.30	89.9
5% s. Calcitonin * 5% leucine raffinose	47	0.31	89.3
5% s. Calcitonin * 20% leucine raffinose	50	0.28	82.9
5% s. Calcitonin * 40% leucine raffinose	48	0.29	82.3
5% s. Calcitonin * 60% leucine raffinose	53	0.22	80.5
5% s. Calcitonin * 80% leucine raffinose	64	0.29	74.5
5% s. Calcitonin 5% tri-leucine raffinose	58	0.46	89
5% s. Calcitonin 20% tri-leucine raffinose	72	0.50	91.1
5% s. Calcitonin 40% tri-leucine raffinose	76	0.46	83.4
5% s. Calcitonin 60% tri-leucine raffinose	84	0.49	94.3
5% s. Calcitonin 80% tri-leucine raffinose	86	0.49	115.2
* Reference Formulations			

[0115] Representative mass median aerodynamic diameters were determined for two of the formulations.

Table 10. Mass Median Aerodynamic Diameters of Calcitonin Powders

Formulation	MMAD
5% s. Calcitonin * 20% leucine raffinose	3.39
5% s. Calcitonin 20% tri-leucine raffinose	2.87
* Reference Formulation	

[0116] From the above data, it can be seen that tri-leucine can be used to improve the aerosol properties of dry powder formulations of a wide range of active agents/medicaments for aerosolized delivery to the lung.

[0117] Trileucine provided nearly a 100% improvement in the emitted dose value of a control powder containing salmon calcitonin and raffinose, nearly doubling its ED value from 48% to 86%. Moreover, tri-leucine was more effective in enhancing powder dispersibility than leucine. While a representative formulation containing 80% by weight leucine exhibited an ED value of 64%, formulations containing 60-80% tri-leucine possessed ED values from 84-86%, further indicating the superiority of tri-leucine in significantly enhancing the aerosol performance of dry powders.

Example 5

Aerosol Properties of Antihiotic-Trileucine Dry Powders

[0118] The ability of tri-leucine to improve the dispersibility of antibiotic-containing dry powders was explored.

A. Antibiotic Control Powders Absent Trileucine

[0119] **Ciprofloxacin Powders.** Aqueous solutions containing the components presented in Table 9 were prepared at a total solids content of 1% (w/v). The pH of each solution was determined, and solutions were then spray-dried as described in Example 2 to prepare dry powders.

Table 11.

Batch Number	Quantitative Composition Prior to Spray Drying[1]		Moisture Content	MMA D (μm)	Emitted Dose
(1) 1326-16	Ciprofloxacin hydrochloride DI water solid product: cipro	1136 mg 113 ml 100%	1.4%	2.8	42% (RSD=8)
(2) 1326-29	Ciprofloxacin hydrochloride DI water Sodium hydroxide solid product: cipro	2047 mg 200 ml QS to pH=12 100%	3.2%	4.5	51% (RSD=7)
(3) 1300-MG-7	Ciproiloxacin hydrochloride Methanol DI water solid product:	1995 mg 100 ml 100 ml 100%	1.2%	2.9	33% (RSD=13)

(continued)

Batch Number	Quantitative Composition Prior to Spray Drying[1]	Moisture Content	MMA D (μm)	Emitted Dose
	cipro			

Gentilmicin, Netilmicin Powders.

[0120] Dry powder compositions containing gentamicin or netilmicin were prepared by mixing gentamicin sulfate or netilmycin sulfate and excipient(s) (if used) with a liquid medium to form a solution. The pH of the solution was adjusted as appropriate to facilitate solubilization and/or stabilization of the components in the solution. Quantitative formulations are identified in Table 12 below. The solutions were then spray-dried as described in Example 2 above to yield dry powders. For formulations that utilized organic solvents, a modified Buchi 190 Mini Spray Dryer was used that was supplied with nitrogen as the gas source and equipped with an oxygen sensor and other safety equipment to minimize the possibility of explosion.

Table 12. Gentamicin/Netilmicin Dry Powders

Batch Number	Quantitative Composition		Moisture Content	MMAD (μm)	Emitted Dose
1326-31	Gentamicin sulfate DI water Hydrochloric acid	2076 mg 200 ml QS to pH=5	4.1%[1]	3.0	37% (RSD[3]=6)
1326-32	Gentamicin sulfate DI water Sodium hydroxide	2053 mg 200 ml QS to pH=10	1.1%[1]	2.4	40% (RSD=14)
1300-MG-11	Gentamicin sulfate Ethanol DI water	2012 mg 40 ml 160 ml	4.8%[3]	3.0	45% (RSD=10)
1300-MG-9	Netilmicin Sulfate DI water	1626 mg 163 ml	4.2%	3.2	47% (RSD=8)
1300-MG-1	4 Netilmicin Sulfate Ethanol DI water	1512 mg 30 ml 120 ml	5.1%	2.9	39% (RSD=7)
[1] Determined with Karl-Fischer reagent titrimetric method [2] Determined with thermogravimetric analysis [3] Relative Standard Deviation					

B. Trileucine-Containing Antibiotic Powders

[0121] Aqueous solutions (100 ml total volume) containing antibiotic and tri-leucine at a total solids content of 1% were prepared and the pH of the solutions adjusted to pH 4. The resulting solutions were then spray-dried to produce powders having the relative amounts of antibiotic and tri-leucine indicated in Table 13 below.

Table 13. Antibiotic-Trileucine Dry Powders

Formulation	Yield, %	MMAD, μm	FPD (<3.3 μm)	ED, %
95% Cipro 5% Leu-3	64.2	2.43	0.57	77.7
75% Cipro 25% Leu-3	N.A.	2.65	N.A.	83.0
45% Cipro 55% Leu-3	55.0	2.62	0.48	70.7

(continued)

Formulation	Yield, %	MMAD, μm	FPD (<3.3 μm)	ED, %
95% Gent. 5% Leu-3	61.4	2.15	0.66	75.7
75% Gent. 25% Leu-3	52.0	2.25	0.66	93.9
55% Gent. 45% Leu-3	54.2	2.51	0.51	87.3
95% Netil. 5% Leu-3	62.0	2.08	0.58	82.4
75% Netil. 25% Leu-3	50.0	2.14	0.66	91.3
55% Netil. 45% Leu-3	40.0	2.73	0.49	90.4

[0122] As can be seen from the results in Table 13, the addition of tri-leucine was effective to notably enhance the dispersibility of powders prepared from three different antibiotic compounds from two different antibiotic classes, ciprofloxacin (a quinolone), gentamicin and netilmicin (aminoglycosides). The ED values for ciprofloxacin powders increased from values ranging from 33-51% to values ranging from 71-83%. Similar beneficial results were observed for gentamicin powders, whose ED values were improved from 37-45% to 76-94% by addition of tri-leucine, and for netilmicin, whose ED values improved from 39-47% to 82-91%. The optimal relative amount of tri-leucine was determined for each of the three antibiotic powders and determined to be approximately 25%, i.e., optimal ED values were observed for powders containing 25% by weight tri-leucine relative to antibiotic.

Example 6

Aerosol Properties of Powders Containing Interferon-β in Combination with Trileucine

[0123] The broad applicability of the use of surface active di- and tri-peptides for increasing powder dispersivity was further explored in interferon-β powders. Interferon-β (a type I interferon) is a cytokine with antiviral, antiproiiferative, and immunomodulatory activity.

[0124] Powders containing interferon-β and optionally tri-leucine and/or other excipients (hydroxyethylstarch, HES and raffinose) were prepared as described above. The solids content of the pre-dried solutions was 1%, with the exception of Lot No. RB27, which possessed a solids content of 0.5%. The composition of the final powders is given in Table 14 below.

Table 14. Interferon-β Powders Containing Tri-leucine

Lot #	Comp.	ED, % mean (n=10)	RSD, %	MMAD, μm	FPD, %	Yield, %	% < 5 μm, %
RB19	10% IFN-β 45%Leu-3 45% HES	81	7	3.2	48	56	79
RB21	10% IFN-β 45% Leu-3 45% Raff.	80	6	2.9	46	61	85
RB24	10% IFN-β 90% Leu-3	-	-	-	-	9*	-
RB27	10% IFN-β 67.5% Leu-3 22.5% Raff.	74	4	2.9	49	40	81
RB29	10% IFN-β 45% Leu-3 45% HES	79	5	3.2	41	50	83

(continued)

Lot #	Comp.	ED, % mean (n=10)	RSD, %	MMAD, μm	FPD, %	Yield, %	% < 5 μm, %
RB36	10% IFN-β 22.5% Leu-3 67.5% Raff.	87	3	-	-	61	-
99320 *	10% IFN-β 90% Raff.	64	-	-	-	-	-
*No tests performed due to low yield. * Reference Composition							

[0125] As with the other active-agent containing powders, the addition of tri-leucine to powders composed of interferon-β served to increase the dispersivity and overall aerosol properties of the resulting powder. Although the improvement was not as striking in some of the previous examples, addition of tri-leucine enhanced the ED values of an interferon-β powder from 64% to 74-87%. As in the previous example, it appears that an optimal amount of tri-leucine is around about 22-25% by weight for the IFN-β powder.

Example 7

Factor IX Dry Powders

[0126] Powders containing factor IX, a 55,000 dalton glycoprotein with a modular domain structure and numerous posttranslational modifications, useful in the treatment of hemophilia B, and trileucine and/or other excipient(s), were prepared to further explore the dispersivity-enhancing effects of tri-leucine and other surface active di- and tri-peptides on different medicaments.

[0127] Powders containing Factor IX, both with and without leucine or a leucine-containing dimer or trimer, were prepared as described previously. The solids content of the pre-spray-dried solution was 1 % by weight (w/v). Yields of the spray dried powders ranged from 40 to 60%. The formulations of the dried powders are provided in Table bellow.

Table 15. Factor IX Powders

Formulation	Emitted Dose (RSD)	MMAD
93% Factor IX/7% * NaCitrate	57 (5 %)	-
37% Factor 1X/3% Na * Citrate/60% Leucine	78 (3%)	2.9
56% Factor 1X/4% Na Citrate/40% Trileucine	89 (5%)	2.7
* Reference Formulation		

[0128] The results in Table 15 further support the effectiveness of tri-leucine at significantly improving the dispersibility of dry powder compositions, irrespective of the active agent contained in the composition. Moreover, as in the previous examples, tri-leucine is better than leucine in significantly improving the dispersibility of the composition (from an ED of 57% to 89%), and can achieve such enhancement when used in smaller quantities than leucine.

Example 8

Stability Studies

[0129] The chemical and physical stability of packaged PTH powders under accelerated stability conditions were evaluated on the basis of the change in protein concentration and aerosol properties measured between initial and 3-month time points. PTH-trileucine and PTH-leucine powders were prepared as in Example 2 above.

[0130] Powders were hand-filled in blister packs (BPs). The blister packs were placed in petri dishes (20-60 BPs/dish).

Table 16. Accelerated stability study at 40 °C/Ambient Relative Humiditv

Formulation ID Composition	Packaged Storage Condition no 2nd wrap no dessicant 40°C/ambient RH	%Purity (by area)	% Emitted Dose (rsd)	Fine Particle Dose (FPD) <3.3μm	MMAD (μm)	% Wt. Change (TGA)
R99484 * 30% PTH/ 70%Leucine	initial	97.0	79.6 (3)	0.58	2.5	1.4
	4 weeks	n/a	74.9(5)	n/a	n/a	1.7
	6 weeks	n/a	75.2 (6)	n/a	n/a	n/a
	8 weeks	95.2 *	78.8 (6)	0.55	2.4	2.2
	12 weeks	n/a	78.6 (3)	n/a	n/a	TBD
R99485 30% PTH/ 70% tri-leucine	initial	97.1	79.4	0.45	2.9	2.6
	4 weeks	n/a	75.8	n/a	n/a	2.4
	6 weeks	n/a	81.6	n/a	n/a	n/a
	8 weeks	94,8	81.6	0.44	2.9	2.4
	12 weeks	n/a	out of BP	n/a	out of BP	out of BP
* the chemical stability of the 8 weeks, 40°C/ambient RH sample is similar to the stability of the 6 months, 40°C/dry sample (foiled wrapped w/ desiccants) of a 30% PTH/70% mannitol formulation. * Reference Formulation						

[0131] In looking at the results in Table 16, it can be seen that the trileucine-containing formulation is both chemically and physically stable upon storage, even at temperatures increased over ambient. Specifically, the 30% PTH/70% trileucine powder exhibited minimal degradation of protein over the timecourse of 3 months, while the aerosol performance of the powder remained essentially unchanged.

Example 9

Electron Spectroscopy of Chemical Analysis (ESCA) of Powder Formulations

[0132] ESCA analysis was carried out on certain powder formulations to investigate the surface enrichment of di-leucyl di- or tripeptide in the particles. The relative concentrations of powder components in the bulk powder is provided in the column, "Formulation"; the concentration of each component on the surface of the particles, as determined by ESCA, is provided in the column, "ESCA result".

Table 17. SCal/Raffinose/Leu formulations pH7

Lot No.		Formulation (%w/w)	ESCA result (%w/w)
R99282 *	sCal	5	53
pH7	Leucine	0	
	Raffinose	95	47
R99283 *	sCal	5	11
pH7	Leucine	5	52
	Raffinose	90	38
R99284 * pH7	sCal	5	39
	Leucine	20	28
	Raffinose	75	33

(continued)

Lot No.		Formulation (%w/w)	ESCA result (%w/w)
R99286 * pH7	Scal	5	26
	Leucine	80	64
	Raffinose	15	9
* Reference Formulations			

Table 18. Leucyl-Peptide/Raffinose formulations

Lot No.		Formulation (%w/w)	ESCA result (%w/w)
R99337	Leucine-2	5	28.7
oH7	Raffinose	95	71.3
R99338	Leucine-2	20	44.1
pH7	Raffinose	80	55.9
R99339	Leucine-2	60	94.9
pH7	Raffinose	40	5.1
R99340 pH7	Leucine-3	20	97.1
	Raffinose	80	2.9
R99342 * pH7	Alanine-3	20	41.3
	Raffinose	80	58.7
* Reference Formulation			

Table 19. SCal/Raffinose/Leu-3 formulations pH4

Lot No.		Formulation (%w/w)	ESCA result (%w/w)
R99435 pH4	Scal	5	36.6
	Leucine-3	0	0
	Raffinose	95	63.4
	Scal	5	17.9
pH4	Leueine-3	5	7.0
	Raffinose	90	75.1
R99437	Scal	5	46.4
pH4	Leucine-3	20	24.2

(continued)

Lot No.		Formulation (%w/w)	ESCA result (%w/w)
	Raffinose	75	29.4
R99438 pH4	Scal	5	22.7
	Leucine-3	40	74.8
	Raffinose	55	2.5
R99439 pH4	Scal	5	16.4
	Leucine-3	60	81.6
	Raffinose	35	2.0

[0133] The above results indicate that powders containing a surface active material are enriched at the surface in concentration of surface active material. Surface enrichment of di- or trileucine is observed for both the non-active agent containing powders in Table 18 and for the s. calcitonin powders in Table 19.

[0134] Although the ESCA results for the calcitonin powders are subject to some variability (this is due to the difficulty of separating out surface concentration contributions by components having the same atom within their structures i.e., calcitonin vs. trileucine), the overall trend observed supports the finding of powders in which the surface concentration of the di-leucyl di- or tripeptide is greater than that in the bulk powder.

Claims

1. A dry powder composition composed of particles which have a mass median diameter (MMD) of less than 20 μm comprising an active agent and a di- or tripeptide selected from dileucine, leu-leu-gly, leu-leu-ala, leu-leu-val, leu-leu-leu, leu-leu-ile, leu-leu-met, leu-leu-pro, leu-leu-phe, leu-leu-trp, leu-leu-ser, leu-leu-thr, leu-leu-cys, leu-leu-tyr, leu-leu-asp, leu-leu-glu, leu-leu-lys, leu-leu-arg, leu-leu-his, leu-leu-nor, leu-gly-leu, leu-ala-leu, leu-val-leu, leu-ile-leu, leu-met-leu, leu-pro-leu, leu-phe-leu, leu-trp-leu, leu-ser-leu, leu-thr-leu, leu-cys-leu, leu-tyr-leu, leu-asp-leu, leu-glu-leu, leu-lys-leu, leu-arg-leu, and leu-nor-leu.

2. The composition of claim 1, wherein the composition is suitable for delivery to the lung or deep lung by inhalation.

3. The composition of claim 1, wherein the dry powder composition comprises dry powder particles, wherein the concentration of the tripeptide on the surface of the particles is greater than in the bulk powder.

4. The composition of claim 1, wherein the tripeptide is present in an amount effective to increase the emitted dose of the composition over the emitted dose of the composition absent the tripeptide.

5. The composition of claim 1, wherein the tripeptide is present in an amount ranging from 1 wt% to 99 wt%.

6. The composition of claim 5, wherein the tripeptide is present in an amount ranging from 5 wt% to 75 wt%.

7. The composition of claim 6, wherein the tripeptide is present in an amount ranging from 5 wt% to 50 wt%.

8. The composition of claim 1, further comprising a pharmaceutically acceptable excipient.

9. The composition of claim 8, wherein the excipient is selected from carbohydrate, amino acid, peptide, protein, organic acid salt, and polymer.

10. The composition of claim 1, wherein the composition has an emitted dose of at least 30%.

11. The composition of claim 1, wherein the composition has an emitted dose of at least 45%.

12. The composition of claim 1, wherein the tripeptide comprises two leucines and an amino acid selected from leucine (leu), valine (val), isoleucine (ile), tryptophan (trp)anine (ala), methionine (met), phenylalanine (phe), tyrosine (tyr), histidine (his), and proline (pro).

13. The composition of claim 12, wherein the tripeptide is trileucine.

14. The composition of claim 1, wherein the active agent comprises insulin and the tripeptide comprises trileucine.

15. The composition of claim 1, wherein the composition comprises particles having an MMD of less than 10 microns.

16. The composition of claim 1, wherein the composition comprises particles having an MMD of less than 4.0 microns.

17. The composition of claim 1, wherein the composition comprises particles having an MMAD of less than 10 microns.

18. The composition of claim 1, wherein the composition comprises particles having an MMAD of less than about 4 microns.

19. The composition of claim 1, wherein the tripeptide has a glass transition temperature greater than 40 °C.

20. The composition of claim 1, wherein the composition has stable dispersivity over time, as **characterized by** a drop in emitted dose of no more than 10% when the composition is stored under ambient conditions for a period of three months.

21. The composition of claim 1, wherein the composition has chemical stability over time, as **characterized by** degradation of less than 5 wt% of the active agent upon storage of the composition under ambient conditions for a period of three months.

22. The composition of claim 1, wherein the active agent is selected from insulin, cyclosporin, parathyroid hormone, follicle stimulating hormone, VLA-4 inhibitors, interleukin- 4R thrombopoietin, c-peptide, amylin, pro-insulin, interleukin-1, interleukin-2, alpha-1-antitrypsin, budesonide, human growth hormone, growth hormone releasing hormone, interferon alpha, interferon beta, growth colony stimulating factor, keratinocyte growth factor, glial growth factor, tumor necrosis factor, leutinizing hormone releasing hormone, calcitonin, low molecular weight heparin, somatostatin, respiratory syncytial virus antibody, erythropoietin, Factor VIII, Factor IX, ceredase, cerezyme and analogues, agonists and antagonists thereof

23. The composition of claim 1, comprising particles having a bulk density ranging from 0.1 grams per cubic centimeter to 10 grams per cubic centimeter.

24. The dry powder composition of claim 23, comprising particles having a bulk density ranging from 0.5 grams per cubic centimeter to 2.0 grams per cubic centimeter.

25. A method for increasing the aerosol performance of an active agent-containing formulation suitable for administration to the lung, the method comprising:

incorporating di- or tripeptide comprising at least two leucines into a formulation comprising an active agent, to thereby form a composition composed of particles which have a mass median diameter (MMD) of less than 20 μm comprising the active agent and the di- or tripeptide,
whereby as a result of the incorporating, the emitted dose of the composition is increased over the emitted dose of a composition having the same components but absent said di- or .. tripeptide.

26. The method of claim 25, wherein the incorporating comprises incorporating a dipeptide where the dipeptide is dileucine.

27. The method of claim 25, wherein the incorporating comprises incorporating a tripeptide comprising two leucines and an amino acid selected from leucine (leu), valine (val), isoleucine (ile), tryptophan (trp), alanine (ala), methionine (met), phenylalanine (phe), tyrosine (tyr), histidine (his), and proline (pro).

28. The method of claim 25, wherein a pharmaceutically acceptable excipient is also incorporated into the formulation.

29. The method of claim 25, wherein the emitted dose of the formulation is increased by at least 5% over that of a formulation having the same components and absent the di- or tripeptide.

30. The method of claim 25, wherein the emitted dose of the formulation is increased by at least 10% over that of a formulation having the same components and absent the di- or tripeptide.

31. The method of claim 25, wherein the formulation has an emitted dose of at least 30%.

32. The method of claim 25, wherein the active agent is selected from insulin, cyclosporin, parathyroid hormone, follicle stimulating hormone, VLA-4 inhibitors, interleukin-4R, thrombopoietin, c-peptide, amylin, pro-insulin, interleukin-1, interleukin-2, alpha-1-antitrypsin, budesonide, human growth hormone, growth hormone releasing hormone, interferon alpha, interferon beta, growth colony stimulating factor, keratinocyte growth factor, glial growth factor, tumor necrosis factor, leutinizing hormone releasing hormone, calcitonin, low molecular weight heparin, somatostatin, respiratory syncytial virus antibody, erythropoietin, Factor VIII, Factor IX, ceredase, cerezyme and analogues, agonists and antagonists thereof

33. The method of claim 25, wherein the formulation is a liquid composition suitable for aerosolized administration to the lung.

34. The method of claim 25, wherein the formulation is a dry composition suitable for aerosolized administration to the lung.

35. Use of a liquid formulation produced by the method of claim 33 for the preparation of a medicament to be delivered to the lungs of a mammalian patient, wherein delivery comprises administering by inhalation the liquid composition.

Patentansprüche

1. Eine Trockenpulverzusammensetzung, zusammengesetzt aus Teilchen, die einen mittleren Massendurchmesser (MMD) von weniger als 20 μm aufweisen, umfassend einen Wirkstoff und ein Di- oder Tripeptid, ausgewählt aus Dileucin, Leu-Leu-Gly, Leu-Leu-Ala, Leu-Leu-Val, Leu-Leu-Leu, Leu-Leu-Ile, Leu-Leu-Met, Leu-Leu-Pro, Leu-Leu-Phe, Leu-Leu-Trp, Leu-Leu-Ser, Leu-Leu-Thr, Leu-Leu-Cys, Leu-Leu-Tyr, Leu-Leu-Asp, Leu-Leu-Glu, Leu-Leu-Lys, Leu-Leu-Arg, Leu-Leu-His, Leu-Leu-Nor, Leu-Gly-Leu, Leu-Ala-Leu, Leu-Val-Leu, Leu-Ile-Leu, Leu-Met-Leu, Leu-Pro-Leu, Leu-Phe-Leu, Leu-Trp-Leu, Leu-Ser-Leu, Leu-Thr-Leu, Leu-Cys-Leu, Leu-Tyr-Leu, Leu-Asp-Leu, Leu-Glu-Leu, Leu-Lys-Leu, Leu-Arg-Leu und Leu-Nor-Leu.

2. Die Zusammensetzung gemäß Anspruch 1, wobei die Zusammensetzung zur Verabreichung in die Lunge oder tiefe Lunge durch Inhalieren geeignet ist.

3. Die Zusammensetzung gemäß Anspruch 1, wobei die Trockenpulverzusammensetzung Trockenpulverteilchen umfasst, wobei die Konzentration des Tripeptids an der Oberfläche der Teilchen größer als in der Masse des Pulvers ist.

4. Die Zusammensetzung gemäß Anspruch 1, wobei das Tripeptid in einer Menge vorhanden ist, die wirksam ist, die freigesetzte Dosis der Zusammensetzung gegenüber der freigesetzten Dosis der Zusammensetzung ohne das Tripeptid zu erhöhen.

5. Die Zusammensetzung gemäß Anspruch 1, wobei das Tripeptid in einer Menge im Bereich von 1 Gew.-% bis 99 Gew.-% vorhanden ist.

6. Die Zusammensetzung gemäß Anspruch 5, wobei das Tripeptid in einer Menge im Bereich von 5 Gew.-% bis 75 Gew.-% vorhanden ist.

7. Die Zusammensetzung gemäß Anspruch 6, wobei das Tripeptid in einer Menge im Bereich von 5 Gew.-% bis 50 Gew.-% vorhanden ist.

8. Die Zusammensetzung gemäß Anspruch 1, weiterhin umfassend einen pharmazeutisch verträglichen Exzipienten.

9. Die Zusammensetzung gemäß Anspruch 8, wobei der Exzipient aus Kohlenhydraten, Aminosäuren, Peptiden,

Proteinen, Salzen organischer Säuren und Polymeren ausgewählt ist.

10. Die Zusammensetzung gemäß Anspruch 1, wobei die Zusammensetzung eine freigesetzte Dosis von mindestens 30 % aufweist.

11. Die Zusammensetzung gemäß Anspruch 1, wobei die Zusammensetzung eine freigesetzte Dosis von mindestens 45 % aufweist.

12. Die Zusammensetzung gemäß Anspruch 1, wobei das Tripeptid zwei Leucine und eine Aminosäure, ausgewählt aus Leucin (Leu), Valin (Val), Isoleucin (Ile), Tryptophan (Trp), Alanin (Ala), Methionin (Met), Phenylalanin (Phe), Tyrosin (Tyr), Histidin (His) und Prolin (Pro), umfasst.

13. Die Zusammensetzung gemäß Anspruch 12, wobei das Tripeptid Trileucin ist.

14. Die Zusammensetzung gemäß Anspruch 1, wobei der Wirkstoff Insulin umfasst und das Tripeptid Trileucin umfasst.

15. Die Zusammensetzung gemäß Anspruch 1, wobei die Zusammensetzung Teilchen mit einem MMD von weniger als 10 μm umfasst.

16. Die Zusammensetzung gemäß Anspruch 1, wobei die Zusammensetzung Teilchen mit einem MMD von weniger als 4,0 μm umfasst.

17. Die Zusammensetzung gemäß Anspruch 1, wobei die Zusammensetzung Teilchen mit einem MMAD von weniger als 10 μm umfasst.

18. Die Zusammensetzung gemäß Anspruch 1, wobei die Zusammensetzung Teilchen mit einem MMAD von weniger als etwa 4 μm umfasst.

19. Die Zusammensetzung gemäß Anspruch 1, wobei das Tripeptid eine Glasübergangstemperatur von mehr als 40 °C aufweist.

20. Die Zusammensetzung gemäß Anspruch 1, wobei die Zusammensetzung eine zeitlich stabile Dispersität, **gekennzeichnet durch** einen Abfall in der freigesetzten Dosis von nicht mehr als 10%, aufweist, wenn die Zusammensetzung unter Umgebungsbedingungen über einen Zeitraum von drei Monaten gelagert wird.

21. Die Zusammensetzung gemäß Anspruch 1, wobei die Zusammensetzung eine chemische Stabilität im Zeitablauf, **gekennzeichnet durch** einen Abbau des Wirkstoffs von weniger als 5 Gew.-% beim Lagern der Zusammensetzung unter Umgebungsbedingungen über einen Zeitraum von drei Monaten, aufweist.

22. Die Zusammensetzung gemäß Anspruch 1, wobei der Wirkstoff ausgewählt ist aus Insulin, Cyclosporin, Parathyroidhormon, Follikel stimulierendem Hormon, VLA-4-Inhibitoren, Interleukin-4R, Thrombopoietin, c-Peptid, Amylin, Proinsulin, Interleukin-1, Interleukin-2, alpha-1-Antitrypsin, Budesonid, menschlichem Wachstumshormon, Wachstumshormon freisetzendem Hormon, Interferon-alpha, Interferon-beta, growth colony-stimulierendem Faktor, Keratinozyten-Wachstumsfaktor, glialem Wachstumsfaktor, Tumornekrosefaktor, luteinisierendes Hormon freisetzendem Hormon, Calcitonin, Heparin von niedrigem Molekulargewicht, Somatostatin, RS-Virusantikörper, Erythropoietin, Faktor VIII, Factor IX, Ceredase, Cerezyme und Analoga, Agonisten und Antagonisten davon.

23. Die Zusammensetzung gemäß Anspruch 1, umfassend Teilchen mit einer Schüttdichte im Bereich von 0,1 Gramm pro Kubikzentimeter bis 10 Gramm pro Kubikzentimeter.

24. Die Trockenpulverzusammensetzung gemäß Anspruch 23, umfassend Teilchen mit einer Schüttdichte im Bereich von 0,5 Gramm pro Kubikzentimeter bis 2,0 Gramm pro Kubikzentimeter.

25. Ein Verfahren zum Verbessern der Aerosolperformance einer einen Wirkstoff enthaltenden Formulierung, die zur Verabreichung in die Lunge geeignet ist, wobei das Verfahren umfasst:

Einbringen eines Di- oder Tripeptids, umfassend mindestens zwei Leucine, in eine einen Wirkstoff umfassende Formulierung, um **dadurch** eine Zusammensetzung zu bilden, die aus Teilchen zusammengesetzt ist, die einen

mittleren Massendurchmesser (MMD) von weniger als 20 μm aufweisen, und die den Wirkstoff und das Di- oder Tripeptid umfasst, wobei infolge des Einbringens die freigesetzte Dosis der Zusammensetzung gegenüber der freigesetzten Dosis einer Zusammensetzung mit den gleichen Bestandteilen, aber ohne das Di- oder Tripeptid, erhöht ist.

26. Das Verfahren gemäß Anspruch 25, wobei der Einbringungsschritt das Einbringen eines Dipeptids umfasst, wobei das Dipeptid Dileucin ist.

27. Das Verfahren gemäß Anspruch 25, wobei der Einbringungsschritt das Einbringen eines Tripeptids, umfassend zwei Leucine und eine Aminosäure, ausgewählt aus Leucin (Leu), Valin (Val), Isoleucin (Ile), Tryptophan (Trp), Alanin (Ala), Methionin (Met), Phenylalanin (Phe), Tyrosin (Tyr), Histidin (His) und Prolin (Pro), umfasst.

28. Das Verfahren gemäß Anspruch 25, wobei ein pharmazeutisch verträglicher Exzipient ebenfalls in die Formulierung eingebracht wird.

29. Das Verfahren gemäß Anspruch 25, wobei die freigesetzte Dosis der Formulierung um mindestens 5 % gegenüber der einer Formulierung mit den gleichen Bestandteilen und ohne das Di- oder Tripeptid erhöht ist.

30. Das Verfahren gemäß Anspruch 25, wobei die freigesetzte Dosis der Formulierung um mindestens 10 % gegenüber der einer Formulierung mit den gleichen Bestandteilen und ohne das Di- oder Tripeptid erhöht ist.

31. Das Verfahren gemäß Anspruch 25, wobei die Formulierung eine freigesetzte Dosis von mindestens 30 % aufweist.

32. Das Verfahren gemäß Anspruch 25, wobei der Wirkstoff ausgewählt ist aus Insulin, Cyclosporin, Parathyroidhormon, Follikel stimulierendem Hormon, VLA-4 Inhibitoren, Interleukin-4R, Thrombopoietin, C-Peptid, Amylin, Proinsulin, Interleukin-1, Interleukin-2, alpha-1-Antitrypsin, Budesonid, menschlichem Wachstumshormon, Wachstumshormon freisetzendem Hormon, Interferon-alpha, Interferon-beta, growth colony-stimulierendem Faktor, Keratinozyten-Wachstumsfaktor, glialem Wachstumsfaktor, Tumornekrosefaktor, luteinisierendes Hormon freisetzendem Hormon, Calcitonin, Heparin von niedrigem Molekulargewicht, Somatostatin, RS-Virusantikörper, Erythropoietin, Faktor VIII, Faktor IX, Ceredase, Cerezyme und Analoga, Agonisten und Antagonisten davon.

33. Das Verfahren gemäß Anspruch 25, wobei die Formulierung eine flüssige Zusammensetzung ist, die für die aerosolisierte Verabreichung in die Lunge geeignet ist.

34. Das Verfahren gemäß Anspruch 25, wobei die Formulierung eine Trockenzusammensetzung ist, die für die aerosolisierte Verabreichung in die Lunge geeignet ist.

35. Verwendung einer flüssigen Formulierung, die durch das Verfahren gemäß Anspruch 33 hergestellt wurde, zur Herstellung eines Medikaments zur Verabreichung in die Lungen eines Säugerpatienten, wobei die Verabreichung das Verabreichen durch Inhalieren der flüssigen Zusammensetzung umfasst.

Revendications

1. Composition de poudre sèche composée de particules qui présentent un diamètre moyen en masse (MMD) de moins de 20 μm comprenant un agent actif et un di- ou tri-peptide choisi parmi la dileucine, leu-leu-gly, leu-leu-ala, leu-leu-val, leu-leu-leu, leu-leu-ile, leu-leu-met, leu-leu-pro, leu-leu-phe, leu-leu-trp, leu-leu-ser, leu-leu-thr, leu-leu-cys, leu-leu-tyr, leu-leu-asp, leu-leu-glu, leu-leu-lys, leu-leu-arg, leu-leu-his, leu-leu-nor, leu-gly-leu, leu-ala-leu, leu-val-leu, leu-ile-leu, leu-met-leu, leu-pro-leu, leu-phe-leu, leu-trp-leu, leu-ser-leu, leu-thr-leu, leu-cys-leu, leu-tyr-leu, leu-asp-leu, leu-glu-leu, leu-lys-leu, leu-arg-leu et leu-nor-leu.

2. Composition selon la revendication 1, où la composition est appropriée être délivrée dans le poumon ou le poumon profond par inhalation.

3. Composition selon la revendication 1, où la composition de poudre sèche comprend des particules de poudre sèche, où la concentration du tripeptide sur la surface des particules est supérieure à celle dans la poudre en vrac.

4. Composition selon la revendication 1, dans laquelle le tripeptide est présent dans une quantité efficace pour aug-

menter la dose émise de la composition par rapport à la dose émise de la composition en l'absence du tripeptide.

5. Composition selon la revendication 1, dans laquelle le tripeptide est présent dans une quantité de 1 % en poids à 99 % en poids.

6. Composition selon la revendication 5, dans laquelle le tripeptide est présent dans une quantité de 5 % en poids à 75 % en poids.

7. Composition selon la revendication 6, dans laquelle le tripeptide est présent dans une quantité de 5 % en poids à 50 % en poids.

8. Composition selon la revendication 1, comprenant en outre un excipient acceptable d'un point de vue pharmaceutique.

9. Composition selon la revendication 8, dans laquelle l'excipient est choisi parmi les glucides, les acides aminés, les peptides, les protéines, les sels d'acides organiques et les polymères.

10. Composition selon la revendication 1, où la composition présente une dose émise d'au moins 30 %.

11. Composition selon la revendication 1, où la composition présente une dose émise d'au moins 45 %.

12. Composition selon la revendication 1, dans laquelle le tripeptide comprend deux leucines et un acide aminé choisi parmi la leucine (leu), la valine (val), l'isoleucine (ile), le tryptophane (trp), l'alanine (ala), la méthionine (met), la phénylalanine (phe), la tyrosine (tyr), l'histidine (his) et la proline (pro).

13. Composition selon la revendication 12, dans laquelle le tripeptide est la trileucine.

14. Composition selon la revendication 1, dans laquelle l'agent actif comprend de l'insuline et le tripeptide comprend la trileucine.

15. Composition selon la revendication 1, où la composition comprend des particules ayant un MMD de moins de 10 microns.

16. Composition selon la revendication 1, où la composition comprend des particules ayant un MMD de moins de 4,0 microns.

17. Composition selon la revendication 1, où la composition comprend des particules ayant un MMAD de moins de 10 microns.

18. Composition selon la revendication 1, où la composition comprend des particules ayant un MMAD de moins d'environ 4 microns.

19. Composition selon la revendication 1, dans laquelle le tripeptide a une température de transition vitreuse supérieure à 40 °C.

20. Composition selon la revendication 1, où la composition a une dispersivité stable dans le temps, **caractérisée par** une diminution de la dose émise non supérieure à 10 % quand la composition est stockée dans des conditions ambiantes pendant une période de trois mois.

21. Composition selon la revendication 1, où la composition présente une stabilité chimique dans le temps, **caractérisée par** une dégradation de moins de 5 % en poids de l'agent actif après stockage de la composition dans des conditions ambiantes pendant une période de trois mois.

22. Composition selon la revendication 1, dans laquelle ledit agent actif est choisi parmi l'insuline, la cyclosporine, l'hormone parathyroïdienne, l'hormone de stimulation des follicules, les inhibiteurs de VLA-4, l'interleukine-4R, la thrombopoïétine, le peptide C, l'amyline, la pro-insuline, l'interleukine-1, l'interleukine-2, l'alpha-1-antitrypsine, le budésonide, l'hormone de croissance humaine, l'hormone libératrice de l'hormone de croissance, l'interféron alpha, l'interféron bêta, le facteur stimulant la croissance des colonies, le facteur de croissance des kératinocytes, le facteur

de croissance gliale, le facteur TNF, l'hormone de libération de la lutéinostimuline, la calcitonine, l'héparine de faible poids moléculaire, la somatostatine, les anticorps du virus respiratoire syncytial, l'érythropoïétine, le facteur VIII, le facteur IX, la cérédase, le cérézyme et leurs analogues, leurs agonistes et leurs antagonistes.

23. Composition selon la revendication 1, comprenant des particules ayant une densité apparente de 0,1 gramme par centimètre cube à 10 grammes par centimètre cube.

24. Composition de poudre sèche selon la revendication 23, comprenant des particules ayant une densité apparente de 0,5 gramme par centimètre cube à 2,0 grammes par centimètre cube.

25. Procédé permettant d'augmenter les performances d'aérosol d'une formulation contenant un agent actif appropriée pour être administrée dans le poumon, le procédé comprenant :

l'incorporation d'un di- ou tri-peptide comprenant au moins deux leucines dans une formulation comprenant un agent actif, pour former de cette manière une composition composée de particules qui ont un diamètre moyen en masse (MMD) de moins de 20 μm comprenant l'agent actif et le di- ou le tripeptide,
moyennant quoi, en résultat de l'incorporation, la dose émise de la composition est accrue par rapport à la dose émise d'une composition contenant les mêmes composants, à l'exception dudit di- ou tripeptide.

26. Procédé selon la revendication 25, dans lequel l'incorporation comprend l'incorporation d'un dipeptide où le dipeptide est la dileucine.

27. Procédé selon la revendication 25, dans lequel l'incorporation comprend l'incorporation d'un tripeptide comprenant deux leucines et un acide aminé choisi parmi la leucine (leu), la valine (val), l'isoleucine (ile), le tryptophane (trp), l'alanine (ala), la méthionine (met), la phénylalanine (phe), la tyrosine (tyr), l'histidine (his) et la proline (pro).

28. Procédé selon la revendication 25, dans lequel un excipient acceptable d'un point de vue pharmaceutique est également incorporé dans la formulation.

29. Procédé selon la revendication 25, dans lequel la dose émise de la formulation est accrue d'au moins 5 % par rapport à celle d'une formulation contenant les mêmes composants à l'exception du di- ou tripeptide.

30. Procédé selon la revendication 25, dans lequel la dose émise de la formulation est accrue d'au moins 10 % par rapport à celle d'une formulation contenant les mêmes composants à l'exception du di- ou tripeptide.

31. Procédé selon la revendication 25, dans lequel la formulation a une dose émise d'au moins 30 %.

32. Procédé selon la revendication 25, dans lequel l'agent actif est choisi parmi l'insuline, la cyclosporine, l'hormone parathyroïdienne, l'hormone de stimulation des follicules, des inhibi-teurs de VLA-4, l'interleukine-4R, la thrombo-poïétine, le peptide C, l'amyline, la pro-insuline, l'interleukine-1, l'interleukine-2, l'alpha- 1-antitrypsine, le budésonide, l'hormone de croissance humaine, l'hormone libératrice de l'hormone de croissance, l'interféron alpha, l'interféron bêta, le facteur stimulant la croissance des colonies, le facteur de croissance des kératinocytes, le facteur de croissance gliale, le facteur TNF, l'hormone de libération de la lutéinostimuline, la calcitonine, l'héparine de faible poids moléculaire, la somatostatine, les anticorps du virus respiratoire syncytial, l'érythropoïétine, le facteur VIII, le facteur IX, la cérédase, le cérézyme et leurs analogues, leurs agonistes et leurs antagonistes.

33. Procédé selon la revendication 25, dans lequel la formulation est une composition liquide appropriée pour une administration aérosolisée dans le poumon.

34. Procédé selon la revendication 25, dans lequel la formulation est une composition sèche appropriée pour une administration aérosolisée dans le poumon.

35. Utilisation d'une formulation liquide produite selon le procédé de la revendication 33, pour la préparation d'un médicament destiné à être délivré aux poumons d'un patient mammifère, dans laquelle la délivrance comprend l'administration par inhalation de la composition liquide.

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US 16245199 P **[0001]**
- US 60164236 B **[0001]**
- US 60172769 B **[0001]**
- US 60178383 B **[0001]**
- US 60178415 B **[0001]**
- US 5376386 A, Ganderton **[0005]**
- US 5855913 A, Hanes **[0005]**
- US 5663198 A, Ruel **[0005]**
- US 5985309 A, Edwards **[0005]**
- US 5908639 A, Simpkin **[0005]**
- WO 9703649 A **[0006]**
- WO 9621461 A **[0007]**
- US 5785049 A **[0027] [0080]**
- US 5922675 A **[0039]**
- WO 9632096 A **[0051]**
- WO 9741833 A **[0059] [0104]**
- US 5985248 A, Gordon, M.S., Lord, J.D. **[0062]**
- US 5976574 A, Gordon, M.S. **[0063]**
- US 6001336 A, Gordon, M. **[0064]**
- WO 9829096 A **[0064]**
- US 5654007 A, Johnson, K. **[0065]**
- US 1997 A **[0065]**
- WO 9509616 A, Ahlneck, C. **[0066]**
- WO 1995 A **[0066]**
- US 5458135 A, Patton, J.S. **[0080]**
- US 5740794 A, Smith, A.E. **[0080]**
- WO 9741031 A, Parks, D.J. **[0081]**
- US 3906950 A, Cocozza, S. **[0082]**
- US 4013075 A, Cocozza, S. **[0082]**
- EP 129985 A **[0083]**
- EP 472598 A **[0083]**
- EP 467172 A **[0083]**
- US 5522385 A, Lloyd, L.J. **[0083]**
- US 4668281 A, Virtanen, R. **[0083]**
- US 4667668 A, Wetterlin, K. **[0083]**
- US 4805811 A, Wetterlin, K. **[0083]**
- US 5388572 A, Mulhauser, P. **[0083]**
- US 5320094 A, Laube **[0084]**
- US 5672581 A, Rubsamen, R.M. **[0084]**

Non-patent literature cited in the description

- Remington: The Science & Practice of Pharmacy. Williams & Williams, 1995 **[0056]**
- Physician's Desk Reference. Medical Economics, 1998 **[0056]**
- Spray Drying Handbook. John Wiley & Sons, Inc, 1991 **[0059]**